Applied Microbiology and Biotechnology (2021) 105:35–53
https://doi.org/10.1007/s00253-020-11017-9

MINI-REVIEW

Hairy root culture technology: applications, constraints and prospect

Saikat Gantait[1] · Eashan Mukherjee

Received: 6 October 2020 / Revised: 4 November 2020 / Accepted: 9 November 2020 / Published online: 23 November 2020

Abstract

Hairy root (HR) culture, a successful biotechnology combining in vitro tissue culture with recombinant DNA machinery, is intended for the genetic improvement of plants. This technology has been put to use since the last three decades for genetic advancement of medicinal and aromatic plants and also to harvest the economical products in the form of secondary metabolites that are significantly important for their ethnobotanical and pharmacological properties. It also provides an efficient way out for the quicker extraction and quantification of the valuable phytochemicals. The current review provides an account of the in vitro HR culture technology and its wide-scale applications in the field of research as well as in pharmaceutical industries. Different facets of HR with respect to the culture establishment, phytochemical production as well as research investigations concerning the areas of gene manipulation, biotransformation of the secondary metabolites, phytoremediation, their industrial utilisations and different problems encountered during the application of this technology have been covered in this appraisal. Eventually, an idea has been provided on HR about the recent trends on the progress of this technology that may open up newer prospects in near future and calls for further research and explorations in this field.

Key points

- *Genetic engineering–based HR culture aims towards enhanced secondary metabolite production.*
- *This review explores an insight in the HR technology and its multi-faceted approaches.*
- *Up-to-date ground-breaking research applications and constraints of HR culture are discussed.*

Keywords Biotransformation · Genetic engineering · In vitro regeneration · Phytoremediation · Secondary metabolites

Introduction

Hairy root (HR) culture technology is a novel biotechnological approach that aims towards the genetic and biochemical improvement of various important medicinal plants (Gantait et al. 2020). An array of secondary metabolites is found in different plants with medicinal value. Alkaloids, tannins, flavonoids, phenolics and other aromatic amino acid derivatives constitute the major bulk of these phytobiochemicals. Naturally, these biochemicals are biosynthesised through different metabolic pathways in plants involving different enzymes or their complexes. These secondary metabolites are found in multiple plant parts such as floral buds, leaves, barks, seeds and, most importantly, in the roots. Extraction of these useful biochemicals (that have remarkable pharmacological, medicinal and ethnobotanical uses) through conventional field-grown methods would involve large-scale uprooting of the plants and, hence, inevitable destruction of their habitat, ecosystem and biodiversity. Such approach has pushed these plants towards endangered category. To mitigate this problem, in vitro culture technology could effectively be applied for the large-scale propagation, conservation as well as production of secondary metabolites from these plants (Gantait et al. 2011; Gangopadhyay et al. 2016; Panigrahi et al. 2018; Verma et al. 2018; Mukherjee et al. 2020). In this scenario, HR culture technology also presents itself as a potential solution to the above-mentioned problem via production and enhancement of secondary metabolite content of these plants (Mitra et al. 2020; Das et al. 2020). These HR cultures could be then

Saikat Gantait and Eashan Mukherjee contributed equally to this work.

✉ Saikat Gantait
saikatgantait@yahoo.com

[1] Crop Research Unit (Genetics and Plant Breeding), Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741252, India

further employed in pharmaceutical research and product development.

The early 1980s marks the inception of the in vitro culture studies of this technology (Willmitzer et al. 1982). HR culture technology mainly involves the use of the soil-inhabiting gram-negative bacteria *Agrobacterium rhizogenes* (Fig. 1). Bacterial cells contain a typical plasmid, called '*Ri*-plasmid' (root-inducing plasmid). During the bacterial infection at the wounded parts of plants, the host plant cells are transformed upon integration of the transfer DNA (T-DNA) segments from the bacteria into its genome (Fig. 2) (Chilton et al. 1982). This transformation is effected owing to the presence of different genes (*rol* genes, i.e. *rol* A, *rol* B and *rol* C) in the plasmid of the bacteria. Upon incorporation, the different genes present in the T-DNA are encoded, and as a result, auxin and cytokinins are produced (Fig. 2) that stimulate the production of HR-like outgrowths from the wounded regions (Shanks and Morgan 1999; Sevon and Oksman-Caldentey 2002; Guillon et al. 2006). On the basis of opine production, *A. rhizogenes* strains are of five types: octopine, mannopine, cucumopine, agropine and nopaline (Zhou et al. 1998), of which agropine strains serve as the preferred choice due to their greater root induction ability. Opine synthesising genes are located in the right border of T-DNA. In homology to the *tms1* and *tms2* of *Ti* plasmid, the TR-DNA contains genes for auxin production, viz. *Tms1* and *Tms2* (Rawat et al. 2019). Resultant HR typifies quick proliferative phytohormone-independent growth, increased biomass and stable genetic performance with respect to secondary metabolite production. This bacterium, i.e. *A. rhizogenes*, also employs binary vector systems where the genes could be carried on another plasmid inside the cell (Tepfer 1984; Christey 1997). Further investigations in this field over years have led to diversified applications such as phytoremediation and biotransformation, which are further elaborated in this review.

Techniques involved

For efficient development of HR cultures, various factors like explant type and growth stage, culture conditions, bacterial strains, suitable media for co-cultivations, pH of the medium and carbon source must be mulled over as these factors influence the secondary metabolite biosynthesis. In general, a range of explants like protoplast, leaf, cotyledons, hypocotyls, shoot tips, stem, stalk, storage root and tubers could be employed (Mugnier 1988; Han et al. 1993; Drewes and Staden 1995; Giri et al. 2001b; Krolicka et al.

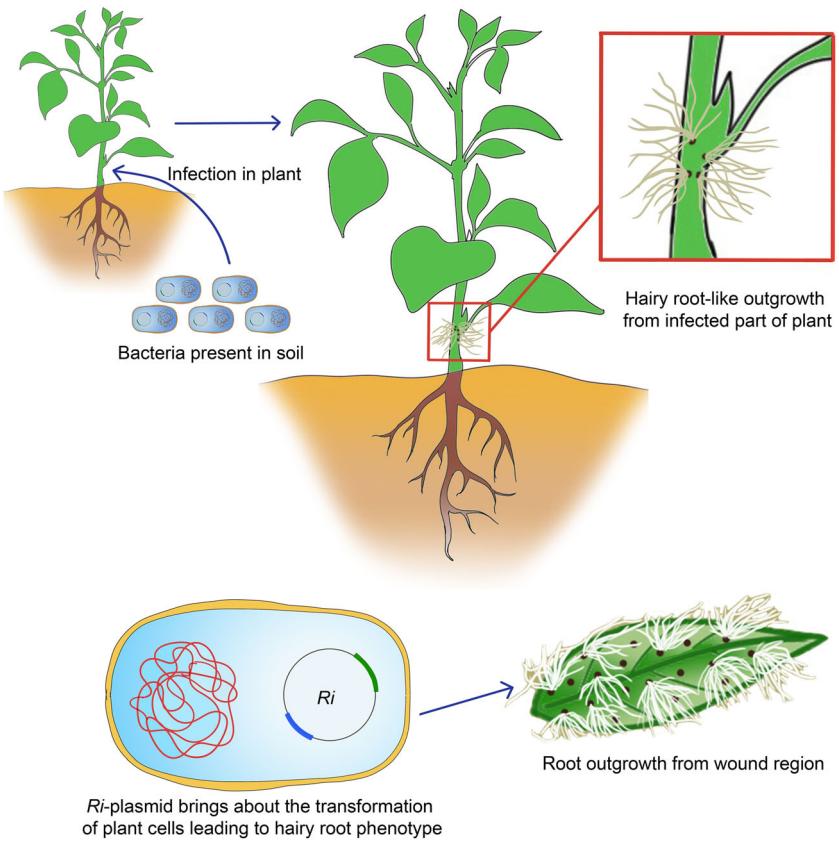

Fig. 1 Diagrammatic representation of *Agrobacterium rhizogenes*–mediated hairy root induction in plants under natural conditions (in vivo) (Source: unpublished diagram of Saikat Gantait)

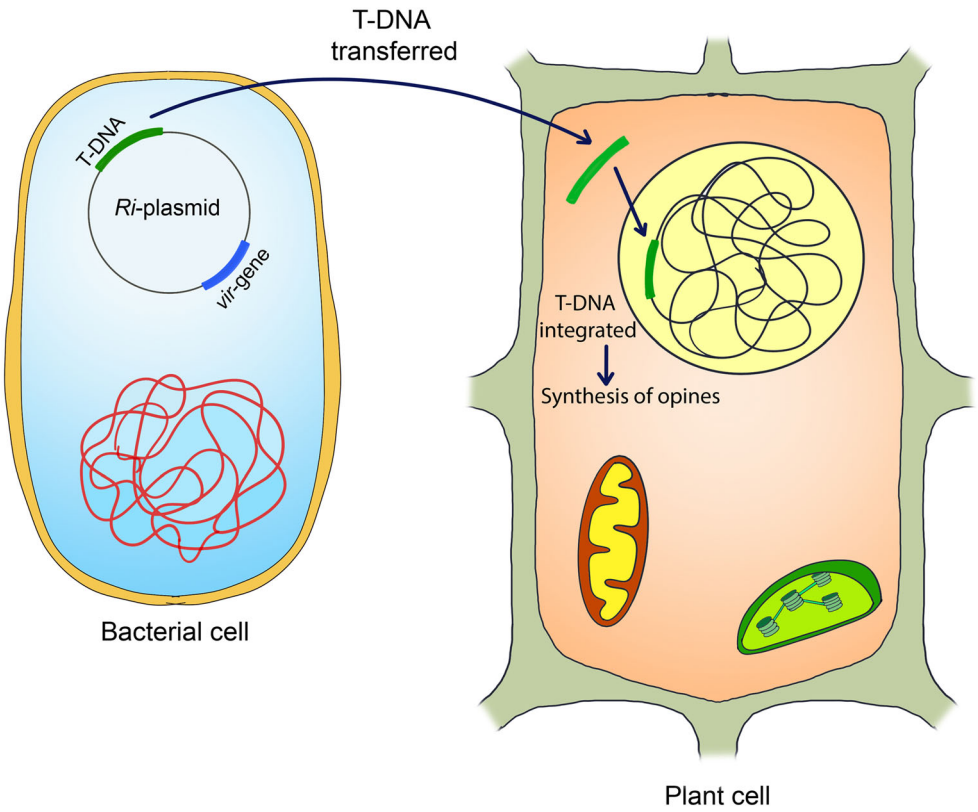

Fig. 2 Diagrammatic representation of the cellular mechanism of *Agrobacterium rhizogenes*–mediated transformation of the host cell (Source: unpublished diagram of Saikat Gantait)

2001; Azlan et al. 2002). In most cases, young and juvenile explants are preferred though, in some cases, it is species dependent (Hu and Du 2006). The explants were initially wounded using a sterile scalpel or needle and then inoculated with bacterial solutions (Fig. 3). Then, explants are co-cultivated in a semisolid medium supplemented with suitable antibiotics such as carbenicillin disodium, cefotaxime sodium, streptomycin sulphate, ampicillin sodium or tetracycline with doses ranging from 100 to 500 μg/ml. This is performed to remove the excess bacteria present on explant surface (Spano et al. 1988; Drewes and Staden 1995; Giri et al. 2001b; Krolicka et al. 2001). Root induction from the wounded places occures within 7 to 30 days. Subsequent subculturing is done in plant growth regulator (PGR)-free medium (Fig. 3). The media supplemented with heavy metal ions of nitrate, ammonia, phosphate and other elicitors (Payne et al. 1987; Toivonen et al. 1991; Christen et al. 1992; Sevon et al. 1992) promote secondary metabolism in the cultures. Culture conditions like light, temperature, pH and different PGR concentrations added to the basal media chiefly influence secondary metabolite production (Christen et al. 1992; Toivonen et al. 1992; Rhodes et al. 1994; Arroo et al. 1995; Bhadra and Shanks 1995; Vanhala et al. 1998; Morgan et al. 2000).

Reporter genes such as *GUS* gene (β-glucuronidase) (Hosoki and Kigo 1994), kanamycin-resistant enzyme encoded by *NPT-II* gene (neomycin phosphotransferase II) (Han et al. 1993; Qin et al. 1994) and green fluorescent protein (*GFP*) encoding gene (in *Catharanthus roseus* L. experiments by Hughes et al. 2002) are the most commonly used ones.

In several published reports, it was mentioned that the improvement in the secondary metabolite production could be effected by a range of different approaches such as precursor feeding, cell permeabilisation and elicitation through the use of different agents like detergents, sonication, calcium chelators, temperature and oxygen stress. The usage of the above-mentioned techniques are found in the reports of Thimmaraju et al. (2003a, b) and Moreno-Valenzuela et al. (2003) describing the production of betalaine from beet root and serpentine from *Catharanthus roseus* HR cultures, respectively. Notably, the usage of chitosan, methyl jasmonate (MeJa) and vanadyl sulphate elicitors in case of *Panax ginseng* stimulated ginsenoside production (Palazon et al. 2003a), whereas *Phytophthora cinnamoni* elicitors stimulated harmine and harmaline in case of *Oxalis tuberose* (Bais et al. 2003). The use of trapping agents such alumina and silica (1:1) in beet root HR cultures in order to enhance betalaine production was reported by Thimmaraju et al. (2004). Cross-species co-culturing method for podophyllotoxin from *Podophyllum hexandrum* using coniferin from *Linum flavum* as precursor was mentioned in the reports of Lin et al. (2003b). Usage of elicitors like $CuSO_4$, MeJa in *Pharbitis nil* for umbelliferone and scopoletin production (Yaoya et al. 2004), *Rhizoctonia bataticola*, β-cyclodextrin and MeJa elicitors in *Solanum tuberosum* for sesquiterpene production was also reported

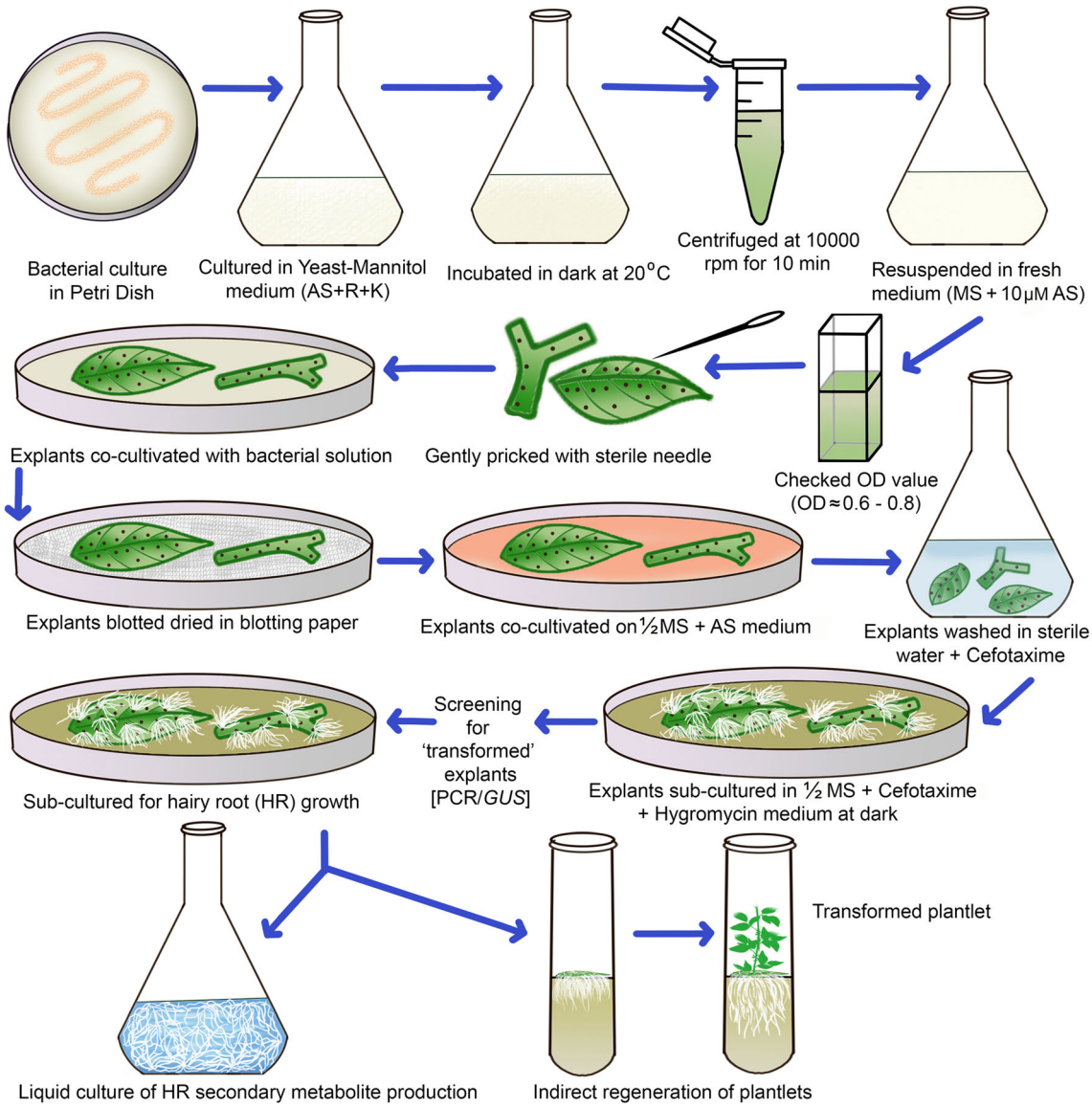

Fig. 3 Diagrammatic representation of the in vitro protocol for hairy root culture establishment using *Agrobacterium rhizogenes* and regeneration of transformed plantlet (Source: unpublished diagram of Saikat Gantait)

(Komaraiah et al. 2003). Similarly, reports of Furze et al. (1991) mention the use of metal ion elicitation by Cu^{2+} and Cd^{2+} in case of *Datura stramonium* for sesquiterpenoid phytoalexin production. Later on, Hu and Du (2006) reported the importance of selection of genetically true high metabolite yielding HR lines. This is due to the ambiguity in proper integration of T-DNA into the plant genome which may lead to variations in the secondary metabolite production.

Applications

Wide-scale research and investigation in this field have facilitated the advancement of varied applications of HR culture technology. Some of the different prospects as explored in the available reports on HR culture technology are mentioned as follows.

Source of important secondary metabolites

Different secondary phytobiochemicals have been isolated using HR culture technology. Some of the notable examples are artemisinin from *Artemisia*, indole alkaloids from *Catharanthus* and *Cinchona*, forskolin from *Coleus*, withanolides from *Withania*, shikonin from *Lithospermum*, diosgenin from *Trigonella*, etc. (Table 1). Likewise, careful maintenance and regular subculturing of *Nicotiana rustica* HR lines stabilised the yield of nicotine at 300 mg/g fresh weight. Similarly, the different published reports regarding the quantification of products of secondary metabolism from

Table 1 Applications of hairy root culture technology in the production of various secondary metabolites (in past two decades)

Plant species	Detection of gene transformation	Secondary metabolite obtained	Detection/quantification method	References
Panax ginseng	PCR analysis using *rol* C, *aux*1, *ags* and *mas*1	Ginsenoside	HPLC	Mallol et al. (2001)
Linum flavum	NM	Coniferin	HPLC	Lin et al. (2003a, b)
Rauvolfia micrantha	PCR analysis using *rol* A primers	Ajmaline, ajmalicine	TLC and HPLC	Sudha et al. (2003)
Papaver somniferum	PCR analysis using *rol* A, *rol* B and *rol* C genes	Morphine, sanguinarine, codeine	HPLC	Le Flem-Bonhomme et al. (2004)
Gynostemma pentaphyllum	PCR analysis using *rol* B primers	Gypenoside	TLC	Chang et al. (2005)
Gmelina arborea	PCR analysis using *rol* B–specific primers	Verbascoside	HPLC	Dhakulkar et al. (2005)
Rauvolfia serpentina	PCR analysis using *rol* A primers	Reserpine	HR culture; NM	Goel et al. (2010)
Bacopa monnieri	PCR detection of TL-DNA and TR-DNA	Bacopasaponin (D and F) and bacopaside (II and V)	HPTLC	Majumdar et al. (2011)
Ageratum conyzoides	PCR analysis using *rol* C primers	β-Farnesene, precocene I and β-caryophyllene	Hydro-distillation	Abdelkader and Lockwood (2011)
Taraxacum officinale	NM	Sesquiterpene lactones	HPTLC	Mahesh and Jeyachandran (2011)
Verbascum xanthophoeniceum	PCR	Verbacoside	LC-APCI-MS analyses	Georgiev et al. (2011)
Gentiana cruciata	PCR analysis using *rol* C primers	Gentiopicroside	HPTLC	Hayta et al. (2011)
Picrorhiza kurroa	PCR analysis revealed the presence of the *rol* B genes	Picrotin and picrotoxinin	HPTLC	Mishra et al. (2011)
Hyoscyamus muticus	PCR analysis using *rol* C primers	Scopolamine and hyoscyamine	GC-MS	Dehghan et al. (2012)
Artemisia annua	PCR	Artemisinin	HPLC	Ahlawat et al. (2012)
Rehmannia glutinosa	PCR analysis using *rol* B and *rol* C primers	Iridoid glycosides and phenylethanoid glycosides	HPLC-ESI-MS	Piątczak et al. (2012)
Clitoria ternatea	Southern blot hybridisation	Taraxerol	HPTLC	Swain et al. (2012)
Panax quinquefolium	PCR analysis	Ginsenosides	HPLC	Kochan et al. (2013)
Scutellaria baicalensis	PCR analysis	Resveratrol	HPLC	Lee et al. (2013)
Dracocephalum kotschyi	PCR detection of *rol* C and *aux*1 genes	Rosmarinic acid	HPLC	Fattahi et al. (2013)
Decalepis hamiltonii	PCR analysis using *rol* C	2-Hydroxy-4-methoxybenzaldehyde	HPLC	Samydurai et al. (2013)
Hypericum perforatum	PCR analysis using *rol* B primers	Xanthone	HPLC	Tusevski et al. (2013)
Gentiana scabra	PCR analysis using *rol B* and *rol C* primers	Loganic acid	HPLC	Huang et al. (2014)
Astragalus membranaceus	PCR amplification of *rol* B, *rol* C and *aux*1 genes	Isoflavonoids	LC-MS/MS	Jiao et al. (2014)
Rauvolfia serpentina	PCR amplification of *rol* B primers	Reserpine	HPLC	Pandey et al. (2014)
Portulaca oleracea	PCR amplification of *rol* B primers	Dopamine	HPLC	Mohghadam et al. (2014)
Polygonum multiflorum	PCR and RT-PCR using *rol* C primers	Pyrogallol, hesperidin, naringenin and formononetin	UPLC	Thiruvengadam et al. (2014)

Table 1 (continued)

Plant species	Detection of gene transformation	Secondary metabolite obtained	Detection/quantification method	References
Tripterygium wilfordii	PCR analysis using *rol* B, *rol* C, *Aux*1, *Vir*G and *Vir*D2 primers	Triptolide and wilforine	HPLC	Zhu et al. (2014)
Rhinacanthus nasutus	PCR analysis and Southern hybridisation analysis	Rhinacanthin	HPLC	Cheruvathur et al. (2015)
Cannabis sativa	NM	Cannabinoids	HPLC and LC-MS	Farag and Kayser (2015)
Stevia rebaudiana	PCR detection of *rol* B and *rol* C genes	Chlorogenic acid	HPLC-MS and HPLC analysis	Fu et al. (2015)
Isatis tinctoria	PCR amplification of *rol* B, *rol* C and *aux*1 genes	Bioactive alkaloids	LC-MS/MS	Gai et al. (2015a, b)
Andrographis paniculata	PCR analysis using *rol* A and *rol* C primers	Andrographolides	HPLC	Marwani et al. (2015)
Gentiana dinarica	GUS assay and PCR analysis	Xanthone	HPLC	Vinterhalter et al. (2015)
Boerhaavia diffusa	PCR analysis using *rol* B and *rol* C primers	Boeravinone B and eupalitin	HPLC	Gupta et al. (2016)
Plumbago rosea	NM	Plumbagin	HPLC	Jose et al. (2016)
Gymnema sylvestre	PCR and RT-PCR	Gymnemic acid	HPTLC and HPLC	Rajashekar et al. (2016)
Abutilon indicum	NM	Quercetin	Shinoda test, Zn-HCl reduction test and ferric chloride and TLC and HPLC	Sajjalaguddam and Paladugu (2016)
Hypericum perforatum	NM	Chlorogenic acid and 3-*p*-coumaroylquinic acid (quercitrin and quercetin isoquercetin), mangiferin	HPLC	Tusevski et al. (2016)
Callerya speciosa	PCR analysis using *rol* B and *rol* C primers, amplification and Southern blot	Polysaccharide	Phenol–sulphuric method	Yao et al. (2016)
Artemisia vulgaris and *Artemisia dracunculus*	NM	Artemisinin	HPTLC-MS	Drobot et al. (2017)
Salvia runcinata	RT-PCR and PCR analysis using *rol* A, *rol* B, *rol* C and *ags* primers	Caffeic acid	GC-MS	Figlan and Makunga (2017)
Vitis vinifera subsp. *sylvesteris*	PCR detection of the *rol* B gene	Resveratrol	HPLC and TLC	Hossieni et al. (2017)
Trifolium pratense	PCR analysis using *rol* A primers	Isoflavones: daidzein, genistein, and biochanin A formononetin	HPTLC	Kumar et al. (2017)
Withania somnifera	PCR analysis using *rol* B and *rol* C primers	Withanolide	HPLC	Shahjahan et al. (2017)
Salvia viridis	PCR analysis using *aux*1, *aux*2, *rol* B and *rol* C primers	Rosmarinic acid	UPLC-PDA-ESI-MS	Grzegorczyk-Karolak et al. (2018)
Ophiorrhiza mungos	PCR analysis using *rol* C and *Vir*C primers	Camptothecin	HPLC and LC-MSMS	Wetterauer et al. (2018)
Sphagneticola calendulacea	Transgenic HR selection via *GUS* staining analysis and PCR analysis	Wedelolactone	HPTLC	Kundu et al. (2018)
Andrographis paniculata	PCR analysis using a *rol* C primer	Andrographolide	HPTLC	Mahobia and Jha (2018)
Linum flavum	PCR analysis of *rol* B, *rol* C	6-Methoxy-podophyllotoxin, podophyllotoxin and deoxypodophyllotoxin	UPLC-HR-MS	Renouard et al. (2018)
Pueraria candollei var. *mirifica*	PCR analysis using a *rol* A primer	Deoxymiroestrol and isoflavonoids	ELISA	Udomsin et al. (2018)

Table 1 (continued)

Plant species	Detection of gene transformation	Secondary metabolite obtained	Detection/quantification method	References
Prosopis farcta	PCR analysis using *rol* C and *Vir*D primers	Flavonoids	LC/MS	Zafari et al. (2018)
Ligularia fischeri Turcz. *f. spiciformis*	PCR analysis using a *rol* C primer	Polyphenolic compounds	UHPTLC	Ansari et al. (2019)
Silene linicola C.C. Gmelin	Not mentioned	20-Hydroxyecdysone (20E), turkesterone and polypodin B	HPLC	Erst et al. (2019)
Ferula pseudalliacea	PCR analysis of the *rol* B gene	Farnesiferol B	HPTLC	Khazaei et al. (2019)
Rehmannia elata	RNA isolation and RT-PCR analysis	Verbascoside and isoverbascoside	UHPTLC	Piątczak et al. (2019)
Trigonella foenum-graecum	PCR analysis using *rol* B primers	Steroidal sapogenin	Gas liquid chromatography	Kohsari et al. (2020)
Corylus avellana	PCR analysis using a *rol* C primer	Paclitaxel	HPTLC	Parizi et al. (2020)

ELISA enzyme-linked immunosorbent assay, *HPLC* high-performance liquid chromatography, *HPTLC* high-performance thin-layer chromatography, *LC-APCI-MS* liquid chromatography–atmospheric pressure chemical ionisation–mass spectrometry, *LC/MS* liquid chromatography/mass spectroscopy, *NM* not mentioned, *PCR* polymerase chain reaction, *RT-PCR* reverse transcriptase polymerase chain reaction, *UPLC-HR-MS* ultra-performance liquid chromatography–high-resolution mass spectrometry, *UPLC-PDA-ESI-MS* ultra-performance liquid chromatography–photodiode array–electrospray ionisation–mass spectrometry, *UPLC* ultra-performance liquid chromatography, *UHPTLC* ultra high-performance thin-layer chromatography

HR cultures mention the isolation of ajmaline, ajmalicine from *Rauvolfia micrantha* (Sudha et al. 2003), verbascoside from *Gmelina arborea* (Dhakulkar et al. 2005), sanguinarine, codeine from *Papaver somniferum* (Le Flem-Bonhomme et al. 2004) and coniferin from *Linum flavum* (Lin et al. 2003a). Recent research investigations have also led to the quantification of different phytochemicals like isoflavones from *Trifolium pratense* (Kumar et al. 2017), crypto tanshinone and tanshinone from *Perovskia abrotanoides* (Ebrahimi et al. 2017), withanolide from *Withania somnifera* (Shahjahan et al. 2017), andrographolides from *Andrographis paniculata* (Mahobia and Jha 2018), flavonoids from *Prosopis farcta* (Zafari et al. 2018), farnesiferol B from *Ferula pseudalliacea* (Khazaei et al. 2019), verbascoside and isoverbascoside from *Rehmannia elata* (Piątczak et al. 2019) and steroidal sapogenin from *Trigonella foenum-graecum* (Kohsari et al. 2020). All of these metabolites have various anti-cancerous, anti-arrhythmic, anti-hypertensive, anti-tumour and multiple other different medicinal properties. This enhancement in the production of different valuable secondary metabolites was accomplished by the introduction of a gene construct encoding an important protein (enzyme) required in the metabolic pathway of the chemical. In some cases, overexpression of the genes also led to the increase in metabolite production. Cultures were fast-growing and easy to maintain and synthesised more than one phytochemical.

Study of gene function

The technique of manipulation of gene construct via which the desired transformation through *A. rhizogenes* is brought about could be further utilised for the studies of gene expression, gene silencing and differential promoter expression levels under different conditions. For example, a promoter induced by a glucocorticoid was utilised to produce the transformed HR cultures of *Catharanthus roseus*. In a glucocorticoid dexamethasone–supplemented medium, the promoter exhibited a reversible dose-regulated response (Hughes et al. 2002). Similarly, Preiszner et al. (2001) in their experimental investigations with HR cultures of soybean reported the differences in the response of the alcohol dehydrogenase promoter fused with the *GUS* gene to different treatments of cold temperature, abscisic acid, low oxygen stress and plant injury.

Gene transfer technology employing *A. rhizogenes* as a vector

The system of binary vector of *A. rhizogenes* can be well utilised to integrate foreign genes, develop gene constructs and transfer them into different plant species. This technique has already been applied in case of *Stylosanthes* (Manners and Way 1989), potato (Visser et al. 1989), cucumber (Trulson et al. 1986) and different crops of Cruciferae (Christey and

Sinclair 1992). Co-integrated *Ri* plasmids with incorporated foreign genes are used for gene transfer in crops like tobacco (Comai et al. 1985), tomato (Morgan et al. 1987) and *Solanum* sp. (Davey et al. 1987). Some of the useful genes like jellyfish protein gene, anthocyanin gene, genes for GUS assay, reporter genes and selectable marker genes like *Hpt* and *NPT II* are transferred (Christey 1997).

Encoding of proteins from related and unrelated taxa

Commercial utilisation of the HR cultures through insertion and gene expression of foreign genes and proteins has been done. Insertion of economically important genes from related or unrelated genera in the transformed root cultures has been achieved. It is noteworthy to mention that viral coat protein genes like that from grapevine mosaic virus were used to transform grapevine HR culture (Torregrosa and Bouquet 1997). Similarly, transformed white clover HR culture was produced upon the transfer of pea Lectin genes (Diaz et al. 1995).

Metabolic engineering

Manoeuvring the genetic construction of the *Ri* plasmid T-DNA of HR-inducing bacteria via incorporation of alien genes between the TL and TR of the *Ri* plasmid that code for the enzymes of the concerned metabolic pathway and then transform the plant cells with this gene construct is metabolic engineering. This method has been lucratively exercised to enhance alkaloid biosynthesis in *Catharanthus roseus* and in different HR cultures of solanaceous plants (Palazon et al. 2003b; Moyano et al. 2003). Production of important biomolecules such as secreted embryonic alkaline phosphatase (SEAP) in human (Gaume et al. 2003), GFP and ricin toxin B (RTB) fusion proteins (from tobacco HR cultures; Medina-Bolivar et al. 2003) and accumulation of poly-3-hydroxybutyrate (from HR cultures of sugar beet; Menzel et al. 2003), solanoside glycoside (from HR culture of *Solanum khasianum*; Putalun et al. 2003), etc., are some of the notable examples mentioned in the different available literature.

Biotransformation

Biotransformation deals with the conversion of naturally available phytochemicals to other biochemicals through structural changes in the original metabolite, employing plant biosystems in the process and thus creating novel and more useful biochemicals suited to the economic use of human beings. This leads to the production of second-generation pharmaceuticals which have enhanced pharmacokinetics, better solubility in biological systems and lesser toxicity. In this context, as per the review report of Banerjee et al. (2012), HR cultures have proven as the beneficial cultures for carrying out this process. These cultures provide low cost, genetic stability and multi-enzyme biosynthetic potential for this process. The different types of biochemical reactions involved in this process in the cultures are hydroxylation, glycosylation, oxidation, condensation, hydrolysis, hydrogenation, acetylations, methylations, esterifications and isomerisation of various exogenously applied substrates as seen in the findings of Giri et al. (2001a) and Ishihara et al. (2003). The most commonly occurring biotransformation reaction is glucosylation or glycosylation. HR cultures of *Coleus forskohlii* effectively biotransformed the ethanol and methanol substrates into their respective β-D-*ribo*-hex-3-ulopyranosides and β-D-glucopyranosides (Li et al. 2003). Similarly, Chen et al. (2008) stated a region-selective glycosylation in HR cultures of *Panax ginseng* where glycosides along with glycosyl esters were produced from the two isomers of hydroxybenzoic acid. Reduction reactions were common in case of HR cultures of *Daucus carota* (Caron et al. 2005) and *Brassica napus* (Orden et al. 2006) with differential stoichiochemistry of their indigenous enzymes to the substrates. Apart from the above two reactions, HR cultures of four members from non-Asteraceae and six members from Asteraceae family exhibited a condensation reaction, producing the dimeric quinone, stilbequinone; though these reactions were rarer compared to the others. In this regard, Flores et al. (1994) reported that the addition of *Pythium aphanidermatum* elicitors to the cultures biotransformed BHT to stilbequinone. Divergence in reaction responses depend upon the mother plant species that is used to initiate the culture. For instance, in the findings of Nunes et al. (2009), HR cultures of *Levisticum officinale* showed differential reaction responses to menthol and geraniol substrates. The HR cultures exhibit acetylation, isomerisation, reduction, cyclisation and oxidation reactions to the addition of geraniol whereas no reaction was seen to methanol addition. Substrates reduced were mainly carbonyl functional group conjugated with aromatic ring.

Apart from the various reaction types involved, other laboratory factors affect the efficiency of biotransformation process. The HR cultures comprising the plant families, viz. Solanaceae, Asteraceae, Campanulaceae and Araliaceae, were more responsive to biotransformation. It was mentioned in the reports that responsiveness to biotransformation was more pronounced at species level than at family level (reviewed in reports of Banerjee et al. 2012). Interestingly, it was pointed out by Nunes et al. (2009) and Faria et al. (2009) that the HR cultures of *Levisticum officinale* and *Anethum graveolens*, having the same taxonomic origin (same family), show differential responses of biotransformation to the same substrate. Considering the strain of the bacterium *A. rhizogenes*, the strains A4, ATCC 15834 and LBA 9402 proved to be more effective than others. Regarding the basal media for biotransformation studies, Murashige and Skoog (1962) medium is the preferred choice. Sucrose was used as a carbon source. In case of *Datura tabula*, favourable results in biotransforming *p*-hydroxybenzyl alcohol into gastrodin were

obtained with supplementation of media with salicylic acid (Peng et al. 2008). Flores et al. (1994) reported 20 h as the time taken for the reactions to complete; though in the findings of Peng et al. (2008), the duration of up to 25 days was reported. It was reported that in most of the cases, the desired products were obtained from the media as well as the roots.

Phytoremediation

The utilisation of plants to tackle environmental pollution by the accumulation and absorption of different heavy metal ions or the polluting contaminants from the substratum and to alter those into nontoxic compounds is called phytoremediation (Suresh and Ravishankar 2004). Through recent research, it has been found that apart from the different in vivo available phytoremediator plants, the transgenically developed HR cultures are also efficient in this detoxification process. In some earlier reports (Agostini et al. 2003; Gujarathi et al. 2005), it is mentioned that different crops like *Helianthus annuus*, *Brassica juncea* and *Cichorium intybus* and their respective HR cultures are efficient in cleansing up pesticides like dichlorodiphenyltrichloroethane (DDT), 2,4-dichlorophenol (2,4-DPC) and other industrial wastes.

There are different reports providing accounts of the phytoremediation technology using HR cultures. The HR is regarded as a model system since this technology allows to study the cellular responses, cell signalling, gene physiological studies and cellular abilities during countering the toxins. Not only this, HR cultures could be utilised in phytomining operations to obtain metal-enriched product from the plant biomass (Boominathan et al. 2004). Under this system, translocation of pollutants to upper parts of the plants do not take place. Quicker proliferation rates and stable genotypic and phenotypic performance of these cultures further make them favourable choices over other cell cultures. Previously mentioned, plant species like *Brassica*, *Helianthus annuus* and *Alyssum* are good bioaccumulators that provide valuable information regarding heavy metal ion accumulation in the rhizosphere and their subsequent management (Eapen et al. 2003; Nedelkoska and Doran 2001; Soudek et al. 2006). To deal with the inorganic and organic pollutants, the genetic architecture of the plants with respect to their ability is altered. This genetic change could be brought about by enhancing the intrinsic proteins that affect the ion accumulation and translocation. This is brought by fortification in the gene expression or by incorporation of related novel genes through biotechnology. This transformation is brought about using suitable gene constructs with plasmids that are easy to manipulate.

Citing an interesting example, Rodríguez-Llorente et al. (2012) reported the utilisation of transgenic HR culture in *Arabidopsis thaliana* to phytoremediate inorganic pollutants through the expression of Cu-binding periplasmic protein (CopC). The organic pollutants were countered via in vitro HR culture of *Atropa belladonna* showing the expression of a rabbit P4502E1 enzyme (Banerjee et al. 2002). Similar investigations were reported in experimental results of Wevar Oller et al. (2005) as well as Sosa Alderete et al. (2009) wherein tobacco and tomato HR cultures expressed a basic peroxidase enzyme to detoxify phenolic compounds more efficiently than natural-type HR. Thus, the concept 'supertransgenic plants through multi-transgene strategy' was put forward (Macek et al. 2008). Hence, the potential of HR culture for further biotechnological interventions is explored.

Production of novel compounds or proteins

An accrual of conjugates of flavonoid glucoside in the HR cultures of *Scutellaria baicalensis* Georgi. was detected when compared to the normally occurring conjugates of glucose as observed in the normal root cultures (Nishikawa and Ishimaru 1997).

Bringing out structural changes in the metabolite

Incorporation and subsequent expression of *Antirrhinum* dihydroflavonol genes in the HR cultures of *Lotus corniculatus* led to changes in the structure of condensed tannin and their increased accumulation in the HR (Bavage et al. 1997). Similar instances of improved metabolite production through genetic changes in the metabolism were mentioned in the reports of Berlin et al. (1993) wherein the *TDC* gene segment from periwinkle (*Catharanthus roseus*) was overexpressed in the HR cultures of *Peganum harmala* that resulted in increased TDC activity. Hashimoto et al. (1993) testified that the constitutive expression of the *H6H* gene transferred from *Hyoscyamus niger* to *Atropa belladonna* led to the conversion of hyoscyamine to scopolamine in the root tissues.

Whole plant regeneration

It has been reviewed by Hu and Du (2006) that through the addition of appropriate PGRs like 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) in the medium, the HR could be induced to produce shoots and somatic embryos. For instance, it was reported that the addition of 7.5–10 mg 2,4-D into the media led to the generation of somatic embryos in the HR cultures of *Astragalus sinicus* (Cho and Wildholm 2002).

Promoting root formation in vegetative propagation

For the successful perpetuation through vegetative propagation, rooting is an important aspect. So, HR culture technology could be utilised to promote rooting in many of the recalcitrant crops like apple, peaches, olive, *Pinus* spp. and *Larix* spp. (McAfee et al. 1993; Bosselut et al. 2011). This is brought

about by the introduction of the Ri plasmid '*rol*' genes of *A. rhizogenes* into the host plant genome (Rugini and Mariotti 1991).

Study of the rhizosphere and culturing of beneficial obligate parasites

By developing an efficient root system in different plants, the study of root morphology, functions and rhizosphere effects as well as plant–microorganism interactions can be performed. Functions and the role of some secondary phytochemicals in influencing the soil bacteria could also be studied. HR cultures can be utilised for the culturing of beneficial fungi like the mycorrhiza. Some of the examples mentioned are *Glomus mosseae* and *Gigaspora margarita* fungi on the HR of *Convolvulus sepium* L. (Mugnier and Musse 1987). These root cultures can also be used for host–pathogen interaction especially the soil-borne pathogens. Cai et al. (1997) were successful in their experiments on developing resistance in sugar beet to beet cyst nematode (*Heterodera schachtii* Schmidt) by transforming susceptible sugar beet HR cultures with the Hs_1^{pro1} gene. Further investigations regarding the outcomes of different other pesticides and chemical on roots and root-interacting microorganisms (both beneficial and harmful) can be done (reviewed by Eapen and Mitra 2001).

Scaling-up process

For up-scaled commercialisation of HR cultures for pharmaceutical implementations, a scaling-up process is important. There has been significant progress in the field of scaling-up process of the HR biosystems with respect to their economic uplift and industry-based research and development. A varied number of bioreactors have surfaced with proficient management facilities and with improved scientific outlook. The exclusive aim of these commercial exploitations is to maximise the benefits derived from the economically invaluable phytochemicals which possess immense ethnobotanical and therapeutic uses. Conventional fermentors are rather unsuitable. There are certain important factors to be kept in mind regarding the setting up of HR bioreactor systems. It has to be borne in mind that prolonged sustenance of cultures in reactor units is vital to the profitable harvest of biochemicals. Various results from different workers show that proper aeration of the culture medium (in case of liquid cultures) is crucial for better circulation, uptake of nutrients and effectual economic product biosynthesis as it optimises oxygen–carbon dioxide balance (Bhojwani and Razdan 1996). HR cultures thrive better in suitable support systems. Varied resources put into use are polyurethane foam (Steingroewer et al. 2013), nylon mesh (Gangopadhyay et al. 2011) and stainless steel base (Srivastava and Srivastava 2012). Angelini et al. (2011) mention the use of nylon mesh in HR bioreactors for phytoremediation studies.

Hence, the following must be kept in mind: distinctive morphological structure of HR, oxygen requirements, etc.; different bioreactors like air-sparged reactors (Taya et al. 1989; Rodriguez-Mendiola et al. 1991); nutrient sprinkle bioreactor (Kuźma et al. 2009); stirred tank reactors (Davioud et al. 1989; Kondo et al. 1989; Hilton and Rhodes 1990; Cardillo et al. 2010; Rahimi et al. 2012); mechanically driven bioreactors, viz. rotary drum reactors and turbine blade reactors (Kondo et al. 1989; Mitchell et al. 2006); immobilised bioreactors like trickle-bed reactors (Flores and Curtis 1992); nutrient mist bioreactor (Huang et al. 2004); pneumatically driven bioreactors, viz. airlift balloon bioreactors (Ali et al. 2007); bubble column reactors (Hilton and Rhodes 1990; Rodriguez-Mendiola et al. 1991; Kwok and Doran 1995; Ludwig-Müller et al. 2008; Georgiev et al. 2012); etc. However, the response to these bioreactors is species specific. For instance, HR cultures of *Artemisia annua* exhibit sustenance for longer period in inner-loop airlift bioreactor (Liu et al. 1998). Apart from this, airlift reactors (in case of *Solanum chrysotrichum*) and mist reactors are also used (Caspeta et al. 2005). In the experimental investigations of Ramakrishnan and Curtis (2004) and Suresh et al. (2005), it was stated that in mist reactors, HR cultures were hung to a mesh support, thereby reducing the amount of culture media and also obtaining the desired phytochemical in concentrated form. The latter method is widely used by the ROOTec Company. As a cost-effective approach, 'rhizosecretion' of the phytochemicals could be achieved by integration of the plant-based hydroponic system with the HR culture system (Gaume et al. 2003).

Computer graphics–based mathematical simulation modelling tactics are deployed to address the various culture-related issues of HR in bioreactors. Srivastava and Srivastava (2006) opined that this mathematical calculative strategy can greatly help researchers in finding the best possible combination of micronutrients and macronutrients for HR growth in bioreactors, leading to time economy and averting resource wastage. Under practical conditions in general due to the dynamicity of the plant cellular organs, exact estimation of the growth kinetics is not possible. Hence to maximise precision, different workers have put forward different models, viz. Patra and Srivastava (2015) in *Artemisia annua* and Thakore et al. (2015) in *Catharanthus roseus*. Mairet et al. (2010) in their investigative reports have recommended different models to characterise HR culture, viz. branching model (based on root differentiation), metabolic model (based on differential metabolic processes) and oxygen-limited growth kinetic model (based on oxygen concentration in the culture).

Of late, bioreactors with novel prototypes have been put into use to improvise the pharmaceutical applications of HR culture as secondary biochemical resources. Some of the

reactor systems worth mentioning are hydraulically driven bioreactors—wave-mixed bioreactors (Huang and McDonald 2012), temporary immersion bioreactors (Ducos et al. 2009), microbioreactors (Diao et al. 2008), ebb-and-flow regime bioreactors (Cuello and Yue 2008) and slug bubble bioreactors (Kantarci et al. 2005).

Constraints encountered

Despite many lucrative advantages, HR culture technology is fraught with many problems and hindrances (Ibañez et al. 2016). General problems encountered during the culturing are as follows.

Probable chromosomal aberrations

Progressive reduction in the chromosome numbers in cultures of *Onobrychis viciifolia* Scop. was noticed over a subculturing period ranging from 4 to 8 months. The chromosome number of normal somatic cells ($2n$ = 28) was found in 4.1% of cells (Hu and Du 2006).

Genetic changes leading to gene suppression

Silencing of gene expression at later stages of subculturing in tryptophan decarboxylase (TDC) and strictosidine synthase (STR) transformed *Cinchona officinalis* L. cultures despite earlier prolific expression levels (Greerlings et al. 1999). An increase in the copy number of transgenes also may lead to poor gene expression levels, leading to less harvest of a desired metabolite as noticed in HR induction experiments of *Catharanthus roseus* transformed with hamster HMGR cDNA producing higher campesterol and serpentine but altered levels of ajmaline and catharanthine amongst the clones (Ayora-Talavera et al. 2002).

Regenerants showing morphological alterations

As per the available literature, the transgenic regenerants exhibit changes like wrinkled and variegated leaves, plagiotrophic rooting, altered apical dominance and poor plant stature (Tepfer 1984; Tayler et al. 1985; Cardarelli et al. 1987; Spano et al. 1988; Hamamoto et al. 1990). Also, leaf asymmetry and shortened shoot length have also been reported. These changes may be genetically occurring due to inaccuracy in transgene insertion and somaclonal variations (Han et al. 1993).

Overexpression of gene might not always give the expected exponential biochemical production

This condition was noticed in the experimental findings of Koehle et al. (2002) in case of HR cultures of *Lithospermum erythrorhizon* Sieb. showing no further increment in shikonin production.

Related species exhibit differential production of secondary metabolite

HR cultures of two related genera (*Datura metel* and *Hyoscyamus muticus*) produce differential tropane alkaloids (Moyano et al. 2003). Also, the background genotypic constitution of the plants may impinge on the transgene expression, as seen in the HR cultures of *L. corniculatus* (Carron et al. 1994).

Problems faced during phytoremediation

Problems of the HR cultures to tackle the fluctuating environmental changes in view of the quantity, volume and form of pollutants were generated out into the environment (Khandare and Govindwar 2015). Also, the results of this technique in field level differ significantly compared to the laboratory conditions (Angelini et al. 2011). Lack of appropriate infrastructure and skilled personnel is also a problem for large-scale operation.

Application with cutting-edge technologies

Over recent years, transcriptome sequencing has surfaced as an innovative biotechnological advancement in improvising the field of HR culture technology with respect to the illumination of the complex internal biosynthetic processes of phytometabolite production in the HR cultures, thereby facilitating their establishment, biofortification and enhanced secondary metabolite extraction. Bolger et al. (2014) and Chaudhary and Sharma (2016) in their reports mention about the boom in the scientific technology due to the advent of these next-generation technologies of polynucleotide sequencing. HR cultures of different plants of medicinal value have been analysed using this technology such as *Panax ginseng* (Cao et al. 2015), *Astragalus membranaceus* (Tuan et al. 2015), *Salvia miltiorrhiza* (Gao et al. 2014; Xu et al. 2015) and *Rehmannia glutinosa* (Wang et al. 2017). The generally used NGS methods were Illumina/Solexa and Roche 454. From the investigations of Yamazaki et al. (2013) in *Ophiorrhiza pumila* HR culture, the information generated from the sequencing of the cellular transcripts using Illumina/Solexa revealed the metabolic mechanism of camptothecin production. Similarly, Illumina techniques

coupled with RT-qPCR were employed in *Catharanthus roseus* to expound the effects of overexpression of anthranilate synthase in the HR culture (Sun et al. 2016). In *Centella asiatica*, it was found that cytochrome P450 hydroxylase and carboxylase are involved in the production of metabolic intermediates which ultimately yield the products asiaticosides and madecassosides (in reports of Kim et al. 2014). In view of this, transcriptome sequencing provides itself as an innovative way out for studying the various metabolism pathways of phytometabolite production in the HR cultures in view of its high-throughput data generation and effective and quicker characterisation of transcriptomic information.

The scaling-up process of the laboratory-based HR cultures requires efficient and meticulous monitoring and supervision of the cultures in the bioreactors so as to harness the invaluable secondary phytobiometabolites. Effective utilisation of the HR cultures under traditional methods are arduous, time consuming and prone to mismanagement. Hence, in sight of this, modern methods based on in silico simulation modelling are developed to speed up and ease out the pharmaceutical utilisations of the HR cultures. Amongst the various recently published works, different models utilised for HR culture are Box–Behnken design (BBD) in *Isatis tinctoria* for secondary metabolite studies (Gai et al. 2015a, b), artificial neural network systems in *Rauwolfia serpentina* (Mehrotra et al. 2013) for biosystem productivity studies and in *Artemisia annua* (Osama et al. 2013) for evaluation studies on the influences of different bioreactors on HR growth and agent-based modelling in *Beta vulgaris* to study the root morphological parameters (Lenk et al. 2014). Most of the modelling studies aim at evaluation of various growth factors and subsequent setting up of an optimised protocol for HR cultures in bioreactors. In *Catharanthus roseus*, statistical design was used to study media condition effects on HR development (Bhadra and Shanks 1995). Similar mathematical modelling method was practised in *Azadirachta indica* HR to infer about the biomass growth of HR culture affected by differential oxygen and mass transfer rates (Palavalli et al. 2012). Morphological assessments centred on root branching (Kim et al. 1995) and root elongation (Bastian et al. 2008) studies were also done. Regulation of mist in the mist reactors is a crucial factor. Both longer and shorter durations of mist lead to nutrient choking. Ranjan et al. (2009), in their investigations, formulated a mathematical way-out model to optimise the mist bioreactor specifications. An up-to-date response surface methodology (RSM) technology combining statistical and mathematical method has been developed. In the published reports of Amdoun et al. (2009, 2010), this modelling tool finds its application in *Datura stramonium* HR culture for estimating the influences of media composition and metal ion media additives for hyoscyamine enhancement. Another dependable and noteworthy technique is the genetic algorithm technology. Arab et al. (2016) attested in their reports that this computer science–based methodology utilises the principle of survival of the fittest and natural selection which is experimentally achieved through stochastic sampling. Using population parameters, this heuristic technique finds remedies to problems of HR biosystem growth limitations such as media optimisation and root biomass–affecting factors. In silico image analysis tool facilitates researchers to develop a two-dimensional image diagram of HR. In this context, a number of software are available such as WinRHIZO, ROOTEDGE and PetriCam. Findings of Flavel et al. (2017) demonstrate an improvement in the precision of their experiments upon using this tool. Lenk et al. (2012) have testified that by using this technology, the secondary phytobiometabolite production can be determined through root morphology surveillance. Thus, it is seen that these computer-defined approaches help researchers in studying the growth patterns and morpho-physiological responses of the HR systems.

Genome editing has become more lucrative in recent times with the discovery of CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated nuclease 9) technology. This biotechnological tool along with metabolic engineering has found profitable utilisations in HR cultures, enabling researchers to study the gene functions and related biometabolite production pathways. Published results of Ron et al. (2014) mentioned that genetic architecture of HR cultures of tomato was amended, employing this technique, wherein varied deletion and insertion mutations were observed in the *SHR* (*SHORTROOT*) gene which led to phenotypic changes in the HR. Similar gene-editing exploits were carried out in soybean *SHR* and *FE12* genes present in situ along with foreign *bar* gene segment (Cai et al. 2015). Jacobs et al. (2015) in their findings in soybean state that this genetic engineering tool can also be applied to make amends in the genes in homeologous chromosomes in polyploids. The target gene *CPS1* in *Salvia miltiorrhiza* was metabolically engineered through CRISPR/Cas9 using AtU6-26SK, 35S-Cas9-SK and pCambia1300 vectors (Li et al. 2017).

Conclusion and prospects

HR culture technology has been developed into an exciting biotechnological tool for researchers. Despite the occurrence of problems like chromosomal changes and fluctuating harvest of desired biochemical, the cultures are easily manageable, genetically stable than the cell suspension cultures and fast proliferating and provide a lot of pharmaceutical exploration. It also provides for the protection of the plants of medicinal value with respect to their demand for the biosynthesis of invaluable medicinal products by acting as an effectual way out for the secondary metabolite production. Regarding the commercial approaches, a more scientific outlook for cost-

effectiveness and sustained production is required. A lot of research and investigation have already undergone in this concerned sector of biotechnology spanning over the past 25 years. Various aspects like the metabolic engineering, large-scale secondary metabolite production and their enhancement through elicitation studies, studies on host–microorganism interactions through roots, genetic transformation, applying techniques of phytoremediation and biotransformation to produce novel biomolecules, utilising novel biotechnological tools like transcriptome sequencing studies, in silico simulation modelling technologies, mathematical modelling for HR biosystem studies and CRISPR-Cas9 technology for enhanced phytometabolite synthesis were dealt with. Despite extensive research, a lot still requires to be explored to extract the wholesome prospects of HR cultures for crop improvement.

Acknowledgements The authors acknowledge the e-library assistance from the Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India, and are further thankful to the anonymous reviewer(s) and the editor of this article for their critical comments and suggestions on the manuscript.

Authors' contribution SG conceived the idea of the review. SG and EM surveyed the literature and drafted the initial manuscript. SG scrutinised and corrected the manuscript to its final version. Both the authors read and approved the final version of the manuscript prior to its submission.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Abdelkader MAS, Lockwood GB (2011) Volatile oils from the plant and hairy root cultures of *Ageratum conyzoides* L. Nat Prod Res 25:909–917

Agostini E, Coniglio MS, Milrad SR, Tigier HA, Giulietti AM (2003) Phytoremediation of 2,4-dichlorophenol by *Brassica napus* hairy root cultures. Biotechnol Appl Biochem 37:139–144

Ahlawat S, Saxena P, Ram M, Alam P, Nafis T, Mohd A, Abdin MZ (2012) Influence of *Agrobacterium rhizogenes* on induction of hairy roots for enhanced production of artemisinin in *Artemisia annua* L. plants. Afr J Biotechnol 11:8684–8691

Ali MB, Hahn EJ, Paek KY (2007) Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in *Panax ginseng* bioreactor root suspension cultures. Molecules 12:607–621

Amdoun R, Khelifi L, Khelifi-Slaoui M, Amroune S, Asch M, Assaf-Ducrocq C, Gontier E (2010) Optimization of the culture medium composition to improve the production of hyoscyamine in elicited *Datura stramonium* L. hairy roots using the response surface methodology (RSM). Int J Mol Sci 11:4726–4740

Amdoun R, Khelifi L, Khelifi-Slaoui M, Amroune S, Benyoussef EH, Thi DV, Assaf-Ducrocq C, Gontier E (2009) Influence of minerals and elicitation on *Datura stramonium* L. tropane alkaloid production: modelization of the *in vitro* biochemical response. Plant Sci 177:81–87

Angelini VA, Orejas J, Medina MI, Agostini E (2011) Scale up of 2,4-dichlorophenol removal from aqueous solutions using *Brassica napus* hairy roots. J Hazard Mater 185:269–274

Ansari MA, Chung IM, Rajakumar G, Alzohairy MA, Almatroudi A, Khanna VG, Thiruvengadam M (2019) Evaluation of polyphenolic compounds and pharmacological activities in hairy root cultures of *Ligularia fischeri* Turcz. *f. spiciformis* (Nakai). Molecules 24:1586

Arab MM, Yadollahi A, Shojaeiyan A, Ahmadi H (2016) Artificial neural network genetic algorithm as powerful tool to predict and optimize *in vitro* proliferation mineral medium for G × N15 rootstock. Front Plant Sci 7:1526

Arroo RRJ, Develi A, Meijers H, Van de Westerlo E, Kemp AK, Croes AF (1995) Effect of exogenous auxin on root morphology and secondary metabolism in *Tagetes patula* hairy root cultures. Physiol Plant 93:233–240

Ayora-Talavera T, Chappell J, Lozoya-Gloria E, Loyola-Vargas VM (2002) Overexpression in *Catharanthus roseus* hairy roots of a truncated hamster 3-hydroxy-3-methylglutaryl-CoA reductase gene. Appl Biochem Biotechnol 97:135–145

Azlan GJ, Marziah M, Radzali M, Johari R (2002) Establishment of *Physalis minima* hairy roots culture for the production of physalins. Plant Cell Tissue Organ Cult 69:271–278

Bais HP, Vepachedu R, Vivanco JM (2003) Root specific elicitation and exudation of fluorescent [beta]-carbolines in transformed root cultures of *Oxalis tuberosa*. Plant Physiol Biochem 41:345–353

Banerjee S, Shang TQ, Wilson AM, Moore AL, Strand SE, Gordon MP, Doty SL (2002) Expression of functional mammalian P450 2E1 in hairy root cultures. Biotechnol Bioeng 77:462–466

Banerjee S, Singh S, Rahman LU (2012) Biotransformation studies using hairy root cultures – a review. Biotechnol Adv 30:461–468

Bastian P, Chavarría-Krauser A, Engwer C, Jäger W, Marnach S, Ptashnyk M (2008) Modelling *in vitro* growth of dense root networks. J Theor Biol 254:99–109

Bavage AD, Davies IG, Robbins MP, Morris P (1997) Expression of an *Antirrhinum* dihydroflavonol reductase gene results in changes in condensed tannin structure and accumulation in root cultures of *Lotus corniculatus* (bird's foot trefoil). Plant Mol Biol 35:443–458

Berlin J, Ruegenhagen NC, Dietze P, Fecker LF, Goddijn OJM, Hoge JHC (1993) Increased production of serotonin by suspension and root cultures of *Peganum harmala* transformed with a tryptophan decarboxylase cDNA clone from *Catharanthus roseus*. Transgenic Res 2:336–344

Bhadra R, Shanks JV (1995) Statistical design of the effect of inoculum conditions on growth of hairy root cultures of *Catharanthus roseus*. Biotechnol Tech 9:681–686

Bhojwani SS, Razdan MK (1996) Tissue culture media. In: Bhojwani SS, Razdan MK (eds) Plant tissue culture: theory and practice. Elsevier, Amsterdam, pp 39–62

Bolger ME, Weisshaar B, Scholz U, Stein N, Usadel B, Mayer KFX (2014) Plant genome sequencing – applications for crop improvement. Curr Opin Biotechnol 26:31–37

Boominathan R, Saha-Chaudhury NM, Sahajwalla V, Doran PM (2004) Production of nickel bio-ore from hyperaccumulator plant biomass: applications in phytomining. Biotechnol Bioeng 86:243–250

Bosselut N, Van Ghelder C, Claverie M, Voisin R, Onesto JP, Rosso MN, Esmenjaud D (2011) *Agrobacterium rhizogenes*-mediated transformation of *Prunus* as an alternative for gene functional analysis in hairy-roots and composite plants. Plant Cell Rep 30:1313

Cai D, Kleine M, Kifle S, Harloff HJ, Sandal NN, Marcker KA, Klein-Lankhorst RM, Salentijn EMJ, Lange W, Steikema WJ, Wyss U, Grundler FMW, Jung C (1997) Positional cloning of a gene for nematode resistance in sugar-beet. Science 275:832–834

Cai Y, Chen L, Liu X, Sun S, Wu C, Jiang B, Han T, Hou W (2015) CRISPR/Cas9-mediated genome editing in soybean hairy roots. PLoS One 10:e0136064

Cao H, Nuruzzaman M, Xiu H, Huang J, Wu K, Chen X, Li J, Jeong JH, Park SJ, Yang F, Luo J, Luo Z (2015) Transcriptome analysis of methyl jasmonate elicited *Panax ginseng* adventitious roots to discover putative ginsenoside biosynthesis and transport genes. Int J Mol Sci 16:3035–3057

Cardarelli M, Spano L, Mariotti D, Mauro ML, Van-Slyus MA, Costantino P (1987) The role of auxin in hairy root induction. Mol Gen Genet 208:457–463

Cardillo AB, Otálvaro AÁM, Busto VD, Talou JR, Velásquez LME, Giulietti AM (2010) Scopolamine, anisodamine and hyoscyamine production by *Brugmansia candida* hairy root cultures in bioreactors. Process Biochem 45:1577–1581

Caron D, Coughlan AP, Simard M, Bernier J, Piche Y, Chenevert R (2005) Stereoselective reduction of ketones by *Daucus carota* hairy root cultures. Biotechnol Lett 27:713–716

Carron TR, Robbins MP, Morris P (1994) Genetic modification of condensed tannin biosynthesis in *Lotus corniculatus*. 1. Heterologous antisense dihydroflavonol reductase down-regulates tannin accumulation in hairy root cultures. Theor Appl Genet 87:1006–1015

Caspeta L, Quintero R, Villarreal ML (2005) Novel airlift reactor fitting for hairy root cultures: developmental and performance studies. Biotechnol Prog 21:735–740

Chang CK, Chang KS, Lin YC, Liu SY, Chen CY (2005) Hairy root cultures of *Gynostemma pentaphyllum* (Thunb.) Makino: a promising approach for the production of gypenosides as an alternative of ginseng saponins. Biotechnol Lett 27:1165–1169

Chaudhary S, Sharma PC (2016) Next generation sequencing-based exploration of genomes and transcriptomes of medicinal plants. Indian J Plant Physiol 21:489–503

Chen X, Zhang J, Liu JH, Yu BY (2008) Biotransformation of p-, m-, and o-hydroxybenzoic acids by *Panax ginseng* hairy root cultures. J Mol Catal B Enzym 54:72–75

Cheruvathur MK, Jose B, Thomas TD (2015) Rhinacanthin production from hairy root cultures of *Rhinacanthus nasutus* (L.) Kurz. In Vitro Cell Dev Biol—Plant 51:420–427

Chilton MD, Tepfer DA, Petit A, David C, Casse-Delbart F, Tempe J (1982) *Agrobacterium rhizogenes* inserts T-DNA into the genomes of the host plant root cells. Nature 295:432–434

Cho HJ, Wildholm JM (2002) Improved shoot regeneration protocol for hairy roots of the legume *Astragalus sinicus*. Plant Cell Tissue Organ Cult 69:259–269

Christen P, Aoki T, Shimomura K (1992) Characteristics of growth and tropane alkaloid production in *Hyoscyamus albus* hairy roots transformed with *Agrobacterium rhizogenes* A4. Plant Cell Rep 11:597–600

Christey MC (1997) Transgenic crop plants using *Agrobacterium rhizogenes* mediated transformation. In: Doran PM (ed) Hairy roots - culture and applications. Hardwood Acad Pub, Australis, pp 99–111

Christey MC, Sinclair BK (1992) Regeneration of transgenic kale (*Brassica oleracea* var. *acephala*), rape (*B. napus*) and turnip (*B. campestris* var. *rapifera*) plants via *Agrobacterium rhizogenes* mediated transformation. Plant Sci 87:161–169

Comai L, Facciotti D, Hiatt WR, Thompson G, Rose RE, Stalker DM (1985) Expression in plants of a mutant *aro*A gene from *Salmonella typhimurium* confers tolerance to glyphosate. Nature 317:741–744

Cuello JL, Yue LC (2008) Ebb-and-flow bioreactor regime and electrical elicitation: novel strategies for hairy root biochemical production. Electron J Integr Biosci 3:45–56

Das A, Sarkar S, Bhattacharyya S, Gantait S (2020) Biotechnological advancements in *Catharanthus roseus* (L.) G. Don. Appl Microbiol Biotechnol 104:4811–4835

Davey MR, Mulligan BJ, Gartland KMA, Peel E, Sargent AW, Morgan AJ (1987) Transformation of *Solanum* and *Nicotiana* species using an R_1 plasmid vector. J Exp Bot 38:1507–1516

Davioud E, Kan C, Hamon J, Tempe J, Husson JP (1989) Production of indole alkaloids by *in vitro* root cultures of *Catharanthus trichophyllus*. Phytochemistry 28:2675–2680

Dehghan E, Häkkinen ST, Oksman-Caldentey KM, Ahmadi FS (2012) Production of tropane alkaloids in diploid and tetraploid plants and *in vitro* hairy root cultures of Egyptian henbane (*Hyoscyamus muticus* L.). Plant Cell Tissue Organ Cult 110:35–44

Dhakulkar S, Ganapathi TR, Bhargava S, Bapat VA (2005) Induction of hairy roots in *Gmelina arborea* Roxb. and production of verbascoside in hairy roots. Plant Sci 169:812–818

Diao J, Young L, Zhou P, Shuler ML (2008) An actively mixed minibioreactor for protein production from suspended animal cells. Biotechnol Bioeng 100:72–81

Diaz CL, Logman TJJ, Stam HC, Kijne JW (1995) Sugar-binding activity of pea lectin expressed in white clover hairy roots. Plant Physiol 109:1167–1177

Drewes FE, Staden JV (1995) Initiation of and solasodine production in hairy root cultures of *Solanum mauritianum* Scop. Plant Growth Regul 17:27–31

Drobot KO, Matvieieva NA, Ostapchuk AM, Kharkhota MA (2017) Study of artemisinin and sugars accumulation in *Artemisia vulgaris* and *Artemisia dracunculus* "hairy" root cultures. Prep Biochem Biotechnol 47:776–781

Ducos JP, Terrier B, Courtois D (2009) Disposable bioreactors for plant micropropagation and mass plant cell culture. In: Eibl R, Eibl D (eds) Disposable bioreactors, Advances in biochemical engineering/biotechnology, vol 115. Springer, Berlin/Heidelberg, pp 89–115

Eapen S, Mitra R (2001) Plant hairy root cultures: prospects and limitations. Proc Indian Natl Sci Acad 67:107–120

Eapen S, Suseelan KN, Tivarekar S, Kotwal SA, Mitra R (2003) Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. Environ Res 91:127–133

Ebrahimi S, Zaker A, Abrishamchi P, Bahrami AR, Ganjeali A, Sodagar N (2017) Hairy root induction and secondary metabolite production in *Perovskia abrotanoides* Karel. J Plant Process Funct 6:17–26

Erst AA, Zibareva LN, Filonenko ES, Zheleznichenko TV (2019) Influence of methyl jasmonate on production of ecdysteroids from hairy roots of *Silene linicola* CC Gmelin. Russ J Bioorg Chem 45: 920–926

Farag S, Kayser O (2015) Cannabinoids production by hairy root cultures of *Cannabis sativa* L. Am J Plant Sci 6:1–10

Faria JMS, Nunes IS, Figueiredo AC, Pedro LG, Trindade H, Barroso JG (2009) Biotransformation of menthol and geraniol by hairy root cultures of *Anethum graveolens*: effect on growth and volatile components. Biotechnol Lett 31:897–903

Fattahi M, Nazeri V, Torras-Claveria L, Sefidkon F, Cusido RM, Zamani Z, Palazon J (2013) A new biotechnological source of rosmarinic acid and surface flavonoids: hairy root cultures of *Dracocephalum kotschyi* Boiss. Ind Crop Prod 50:256–263

Figlan S, Makunga NP (2017) Genetic transformation of the medicinal plant *Salvia runcinata* L. f. using *Agrobacterium rhizogenes*. S Afr J Bot 112:193–202

Flavel RJ, Guppy CN, Rabbi SMR, Young IM (2017) An image processing and analysis tool for identifying and analysing complex plant root systems in 3D soil using non-destructive analysis: Root1. PLoS One 12:e0176433

Flores HE, Curtis WR (1992) Approaches to understanding and manipulating the biosynthetic potential of plant roots. Ann N Y Acad Sci 665:188–209

Flores HE, Dai YR, Freyer AJ, Michaels PJ (1994) Biotransformation of menthol and geraniol by hairy root cultures. Plant Physiol Biochem 32:511–519

Fu X, Yin ZP, Chen JG, Shangguan XC, Wang X, Zhang QF, Peng DY (2015) Production of chlorogenic acid and its derivatives in hairy root cultures of *Stevia rebaudiana*. J Agric Food Chem 63:262–268

Furze JM, Rhodes MJC, Parr AJ, Robins RJ, Withehead IM, Threlfall DR (1991) Abiotic factors elicit sesquiterpenoid phytoalexin production but not alkaloid production in transformed root cultures of *Datura stramonium*. Plant Cell Rep 10:111–114

Gai QY, Jiao J, Luo M, Wang W, Ma W, Zu YG, Fu YJ (2015a) Establishment of high-productive *Isatis tinctoria* L. hairy root cultures: a promising approach for efficient production of bioactive alkaloids. Biochem Eng J 95:37–47

Gai QY, Jiao J, Luo M, Wei ZF, Zu YG, Ma W, Fu YJ (2015b) Establishment of hairy root cultures by *Agrobacterium rhizogenes* mediated transformation of *Isatis tinctoria* L. for the efficient production of flavonoids and evaluation of antioxidant activities. PLoS One 10:e0119022

Gangopadhyay M, Dewanjee S, Bhattacharya S (2011) Enhanced plumbagin production in elicited *Plumbago indica* hairy root cultures. J Biosci Bioeng 111:706–710

Gangopadhyay M, Gantait S, Palchoudhury S, Ali MN, Mondal C, Pal AK (2016) UVC-priming mediated modulation of forskolin biosynthesis key genes against *Macrophomina* root rot of *Coleus forskohlii*- a tissue culture based sustainable approach. Phytochem Lett 17:36–44

Gantait S, Mandal N, Das PK (2011) *In vitro* accelerated mass propagation and *ex vitro* evaluation of *Aloe vera* L. with aloin content and superoxide dismutase activity. Nat Prod Res 25:1370–1378

Gantait S, Mitra M, Chen J-T (2020) Biotechnological interventions for ginsenosides production. Biomolecules 10:538

Gao W, Sun HX, Xiao H, Cui G, Hillwig ML, Jackson A, Wang X, Shen Y, Zhao N, Zhang L, Wang XJ, Peters RJ, Huang L (2014) Combining metabolomics and transcriptomics to characterize tanshinone biosynthesis in *Salvia miltiorrhiza*. BMC Genomics 15:73

Gaume A, Komarnytsky S, Borisjuk N, Raskin I (2003) Rhizosecretion of recombinant proteins from plant hairy roots. Plant Cell Rep 21: 1188–1193

Georgiev MI, Ludwig-Miller J, Alipieva K, Lippert A (2011) Sonication-assisted *Agrobacterium rhizogenes*-mediated transformation of *Verbascum xanthophoeniceum* Griseb. for bioactive metabolite accumulation. Plant Cell Rep 30:859–866

Georgiev VG, Bley T, Pavlov AI (2012) Bioreactors for the cultivation of red beet hairy roots. In: Neelwarne B (ed) Red beet biotechnology. Springer, Boston, pp 251–281

Giri A, Dhingra V, Giri CC, Singh A, Ward OP, Narasu ML (2001a) Biotransformations using plant cells, organ cultures and enzyme systems: current trends and future prospects. Biotechnol Adv 19: 175–199

Giri A, Ravindra ST, Dhingra V, Narasu ML (2001b) Influence of different strains of *Agrobacterium rhizogenes* on induction of hairy roots and artemisinin production in *Artemisia annua*. Curr Sci 81: 378–382

Goel MK, Goel S, Banerjee S, Shanker K, Kukreja AK (2010) *Agrobacterium rhizogenes* mediated transformed roots of *Rauwolfia serpentina* for reserpine biosynthesis. Med Arom Plant Sci Biotechnol 4:8–14

Greerlings A, Hallard D, Martinez CA, Lopes CI, Heijden RV, Verpoorte R (1999) Alkaloid production by a *Cinchona officinalis* 'Ledgeriana' hairy root culture containing constitutive expression constructs of tryptophan decarboxylase and strictosidine synthase cDNAs from *Catharanthus roseus*. Plant Cell Rep 18:191–196

Grzegorczyk-Karolak I, Kuźma Ł, Skała E, Kiss A (2018) Hairy root cultures of *Salvia viridis* L. for production of polyphenolic compounds. Ind Crop Prod 117:235–244

Guillon S, Trémouillaux-Guiller J, Pati PK, Rideau M, Gantet P (2006) Hairy root research: recent scenario and exciting prospects. Curr Opin Plant Biol 9:341–346

Gujarathi NP, Haney BJ, Park HJ, Wickramasinghe SR, Linden JC (2005) Hairy roots of *Helianthus annuus*: a model system to study phytoremediation of tetracycline and oxytetracycline. Biotechnol Prog 21:775–780

Gupta R, Pandey P, Singh S, Singh DK, Saxena A, Luqman S, Bawankule DU, Banerjee S (2016) Advances in *Boerhaavia diffusa* hairy root technology: a valuable pursuit for identifying strain sensitivity and up-scaling factors to refine metabolite yield and bioactivity potentials. Protoplasma 253:1145–1158

Hamamoto H, Boulter ME, Shirsat AH, Croy EJ, Ellis JR (1990) Recovery of morphogenetically normal transgenic tobacco from hairy roots co-transformed with *Agrobacterium rhizogenes* and a binary vector plasmid. Plant Cell Rep 9:88–92

Han KH, Keathley DE, Davis JM, Gordon MP (1993) Regeneration of a transgenic woody legume (*Robinia pseudoacacia* L. black locust) and morphological alterations induced by *Agrobacterium rhizogenes*-mediated transformation. Plant Sci 88:149–157

Hashimoto T, Yun DJ, Yamada Y (1993) Production of tropane alkaloids in genetically engineering root cultures. Phytochemistry 32:712–718

Hayta S, Gurel A, Akgun IH, Altan F, Ganzera M, Tanyolac B, Bedir E (2011) Induction of *Gentiana cruciata* hairy roots and their secondary metabolites. Biologia 66:618–625

Hilton MG, Rhodes MJC (1990) Growth and hyoscyamine production of hairy root cultures of *Datura stramonium* in a modified stirred tank reactor. Appl Microbiol Biotechnol 33:132–138

Hosoki T, Kigo T (1994) Transformation of Brussels sprouts (*Brassica oleracea* var. *gemmifera* Zenk.) by *Agrobacterium rhizogenes* harbouring a reporter, beta-glucuronidase gene. J Jpn Soc Hort Sci 63:589–592 (in Japanese with an English abstract)

Hossieni SM, Bahramnejad B, Baneh HD, Emamifar A, Goodwin PH (2017) Hairy root culture optimization and resveratrol production from *Vitis vinifera* subsp. *sylvesteris*. World J Microbiol Biotechnol 33:67

Hu ZB, Du M (2006) Hairy root and its application in plant genetic engineering. J Integr Plant Biol 48:121–127

Huang SH, Viswakarma RK, Lee TT, Chan HS, Tsay HS (2014) Establishment of hairy root lines and analysis of iridoids and secoiridoids in the medicinal plant *Gentiana scabra*. Bot Stud 55:17

Huang SY, Hung CH, Chou SN (2004) Innovative strategies for operation of mist trickling reactors for enhanced hairy root proliferation and secondary metabolite productivity. Enzym Microb Technol 35: 22–32

Huang TK, McDonald KA (2012) Bioreactor systems for *in vitro* production of foreign proteins using plant cell cultures. Biotechnol Adv 30:398–409

Hughes EH, Hong SB, Shanks JV, San KY, Gibson SI (2002) Characterization of an inducible promoter system in *Catharanthus roseus* hairy roots. Biotechnol Prog 18:1183–1186

Ibañez S, Talano M, Ontañon O, Suman J, Medina MI, Macek T, Agostini E (2016) Transgenic plants and hairy roots: exploiting the potential of plant species to remediate contaminants. New Biotechnol 33:625–635

Ishihara K, Hamada H, Hirata T, Nakajima N (2003) Biotransformation using plant cultured cells. J Mol Catal B Enzym 23:145–170

Jacobs TB, LaFayette PR, Schmitz RJ, Parrott WA (2015) Targeted genome modifications in soybean with CRISPR/Cas9. BMC Biotechnol 15:16

Jiao J, Gai QY, Fu YJ, Ma W, Peng X, Tan SN, Efferth T (2014) Efficient production of isoflavonoids by *Astragalus membranaceus* hairy root cultures and evaluation of antioxidant activities of extracts. J Agric Food Chem 62:12649–12658

Jose B, Silja PK, Pillai DB, Satheeshkumar K (2016) *In vitro* cultivation of hairy roots of *Plumbago rosea* L. in a customized Reaction kettle

for the production of plumbagin—an anticancer compound. Ind Crop Prod 87:89–95

Kantarci N, Borak F, Ulgen KO (2005) Bubble column reactors. Process Biochem 40:2263–2283

Khandare RV, Govindwar SP (2015) Phytoremediation of textile dyes and effluents: current scenario and future prospects. Biotechnol Adv 33:1697–1714

Khazaei A, Bahramnejad B, Mozafari AA, Dastan D, Mohammadi S (2019) Hairy root induction and farnesiferol B production of endemic medicinal plant *Ferula pseudalliacea*. 3. Biotech 9:407

Kim OT, Um Y, Jin ML, Chang YK, Bang KH, Hyun DY, Lee HS, Lee Y (2014) Analysis of expressed sequence tags from *Centella asiatica* (L.) urban hairy roots elicited by methyl jasmonate to discover genes related to cytochrome P450s and glucosyltransferases. Plant Biotechnol Rep 8:211–220

Kim S, Hopper E, Hjortso M (1995) Hairy root growth models: effect of different branching patterns. Biotechnol Prog 11:178–186

Kochan E, Wasiela M, Seinkiewicz M (2013) The production of ginsenosides in hairy root cultures of American Ginseng, *Panax quinquefolium* L. and their antimicrobial activity. In Vitro Cell Dev Biol—Plant 49:24–29

Koehle A, Sommer S, Yazaki K, Ferrer A, Boronat A, Li SM, Heide L (2002) High level expression of chorismate pyruvate-lyase (UbiC) and HMG-CoA reductase in hairy root cultures of *Lithospermum erythrorhizon*. Plant Cell Physiol 43:894–902

Kohsari S, Rezayain M, Niknam V, Mirmasoumi M (2020) Antioxidative enzymes activities and accumulation of steroids in hairy roots of *Trigonella*. Physiol Mol Biol Plants 26:281–288

Komaraiah P, Reddy GV, Reddy PS, Raghavendra AS, Ramakrishna SV, Reddanna P (2003) Enhanced production of antimicrobial sesquiterpenes and lipoxygenase metabolites in elicitor-treated hairy root cultures of *Solanum tuberosum*. Biotechnol Lett 25:593–597

Kondo O, Honda H, Taya M, Kobayashi T (1989) Comparison of growth properties of carrot hairy root in various bioreactors. Appl Microbiol Biotechnol 32:291–294

Krolicka A, Staniszewska I, Bielawski K, Malinski E, Szafranek J, Lojkowska E (2001) Establishment of hairy root cultures of *Ammi majus*. Plant Sci 160:259–264

Kumar MA, Pammi SS, Sukanya MS, Giri A (2017) Enhanced production of pharmaceutically important isoflavones from hairy root rhizoclones of *Trifolium pratense* L. In Vitro Cell Dev Biol—Plant 54:94–103

Kundu S, Salma U, Ali MN, Hazra AK, Mandal N (2018) Development of transgenic hairy roots and augmentation of secondary metabolites by precursor feeding in *Sphagneticola calendulacea* (L.) Pruski. Ind Crop Prod 121:206–215

Kuźma Ł, Bruchajzer E, Wysokińska H (2009) Methyl jasmonate effect on diterpenoid accumulation in *Salvia sclarea* hairy root culture in shake flasks and sprinkle bioreactor. Enzym Microb Technol 44: 406–410

Kwok KH, Doran PM (1995) Kinetic and stoichiometric analysis of hairy roots in a segmented bubble column reactor. Biotechnol Prog 11: 429–435

Le Flem-Bonhomme V, Laurain-Mattar D, Fliniaux MA (2004) Hairy root induction of *Papaver somniferum* var. *album*, a difficult-to-transform plant, by *A. rhizogenes* LBA 9402. Planta 218:890–893

Lee SW, Kim YS, Uddin MR, Kwon DY, Kim YB, Lee MY, Kim SJ, Par SU (2013) Resveratrol production from hairy root cultures of *Scutellaria baicalensis*. Nat Prod Commun 8:609–611

Lenk F, Sürmann A, Oberthür P, Schneider M, Steingroewer J, Bley T (2014) Modeling hairy root tissue growth in *in vitro* environments using an agent-based, structured growth model. Bioprocess Biosyst Eng 37:1173–1184

Lenk F, Vogel M, Bley T, Steingroewer J (2012) Automatic image recognition to determine morphological development and secondary metabolite accumulation in hairy root networks. Eng Life Sci 12: 588–594

Li B, Cui G, Shen G, Zhan Z, Huang L, Chen J, Qi X (2017) Targeted mutagenesis in the medicinal plant *Salvia miltiorrhiza*. Sci Rep 7: 43320

Li W, Koike K, Asada Y, Yoshikawa T, Nikaido T (2003) Biotransformation of low-molecular-weight alcohols by *Coleus forskohlii* hairy root cultures. Carbohydr Res 338:729–731

Lin HW, Kwok KH, Doran PM (2003a) Development of *Linum flavum* hairy root cultures for production of coniferin. Biotechnol Lett 25: 521–525

Lin HW, Kwok KH, Doran PM (2003b) Production of podophyllotoxin using cross-species co-culture of *Linum flavum* hairy roots and *Podophyllum hexandrum* cell suspensions. Biotechnol Prog 19: 1417–1426

Liu CZ, Wang YC, Ouyang F, Ye HC, Li GF (1998) Production of artemisinin by hairy root cultures of *Artemisia annua* L. in bioreactor. Biotechnol Lett 20:266–268

Ludwig-Müller J, Georgiev M, Bley T (2008) Metabolite and hormonal status of hairy root cultures of devil's claw (*Harpagophytum procumbens*) in flasks and in a bubble column bioreactor. Process Biochem 43:15–23

Macek T, Kotrba P, Svatos A, Novakova M, Demnerova K, Mackova M (2008) Novel roles for genetically modified plants in environmental protection. Trends Biotechnol 26:146–152

Mahesh A, Jeyachandran R (2011) *Agrobacterium rhizogenes*-mediated hairy root induction in *Taraxacum officinale* and analysis of sesquiterpene lactones. Plant Biosyst 145:620–626

Mahobia A, Jha Z (2018) Root cultures: *in vitro* conservative method for metabolite extraction from *A. paniculata*. Int J Curr Microbiol App Sci 7:2442–2450

Mairet F, Villon P, Boitel-Conti M, Shakourzadeh K (2010) Modeling and optimization of hairy root growth in fed-batch process. Biotechnol Prog 26:847–856

Majumdar S, Garai S, Jha S (2011) Genetic transformation of *Bacopa monnieri* by wild type strains of *Agrobacterium rhizogenes* stimulates production of bacopa saponins in transformed calli and plants. Plant Cell Rep 30:941–954

Mallol A, Cusido RM, Palazon J, Bonfill M, Morales C, Pinol MT (2001) Ginsenoside production in different phenotypes of *Panax ginseng* transformed roots. Phytochemistry 57:365–371

Manners JM, Way H (1989) Efficient transformation with regeneration of the tropical pasture legume *Stylosanthes humilis* using *Agrobacterium rhizogenes* and a T_1 plasmid binary vector system. Plant Cell Rep 8:341–345

Marwani E, Pratiwi D, Wardhani K, Esyanti R (2015) Development of hairy root culture of *Andrographis paniculata* for *in vitro* adrographollide production. J Med Bioeng 4:446–450

McAfee BJ, White EE, Pelcher LE, Lapp MS (1993) Root induction in pine (*Pinus*) and Larch (*Larix*) spp. using *Agrobacterium rhizogenes*. Plant Cell Tissue Organ Cult 34:53–62

Medina-Bolivar F, Wright R, Funk V, Sentz D, Barroso L, Wilkins TD, Petri W Jr, Cramer CL (2003) A non-toxic lectin for antigen delivery of plant-based mucosal vaccines. Vaccine 21:997–1005

Mehrotra S, Prakash O, Khan F, Kukreja AK (2013) Efficiency of neural network-based combinatorial model predicting optimal culture conditions for maximum biomass yields in hairy root cultures. Plant Cell Rep 32:309–317

Menzel G, Harloff HJ, Jung C (2003) Expression of bacterial poly(3-hydroxybutyrate) synthesis genes in hairy roots of sugar beet (*Beta vulgaris* L.). Appl Microbiol Biotechnol 60:571–576

Mishra J, Bhandari H, Singh M, Rawat S, Agnihotri RK, Mishra S, Purohit S (2011) Hairy root culture of *Picrorhiza kurroa* Royle ex Benth.: a promising approach for the production of picrotin and picrotoxinin. Acta Physiol Plant 33:1841–1846

Mitchell DA, Stuart DM, Hardin MT, Krieger N (2006) Group III: rotating-drum and stirred-drum bioreactors. In: Mitchell D, Berovic M, Krieger N (eds) Solid-state fermentation bioreactors. Springer, Berlin/Heidelberg, pp 95–114

Mitra M, Gantait S, Mandal N (2020) *Coleus forskohlii*: advancements and prospects of *in vitro* biotechnology. Appl Microbiol Biotechnol 104:2359–2371

Mohghadam YA, Piri K, Bahramnejad B, Ghiasvand T (2014) Dopamine production in hairy root cultures of *Portulaca oleracea* (Purslane) using *Agrobacterium rhizogenes*. J Agric Sci Technol 16:409–420

Moreno-Valenzuela OA, Minero-Garcia Y, Chan W, Mayer-Geraldo E, Carbajal E, Loyola-Vargas VM (2003) Increase in the indole alkaloid production and its excretion into the culture medium by calcium antagonists in *Catharanthus roseus* hairy roots. Biotechnol Lett 25: 1345–1349

Morgan AJ, Cox PN, Turner DA, Peel E, Davey MR, Gartland KMA, Mulligan BJ (1987) Transformation of tomato using an R_1 plasmid vector. Plant Sci 49:37–49

Morgan JA, Barney CS, Penn AH, Shanks JV (2000) Effects of buffered media upon growth and alkaloid production of *Catharanthus roseus* hairy roots. Appl Microbiol Biotechnol 53:262–265

Moyano E, Jouhikainen K, Tammela P (2003) Effect of *pmt* gene overexpression on tropane alkaloid production in transformed root cultures of *Datura metel* and *Hyoxcyamus muticus*. J Exp Bot 54:203–211

Mugnier J (1988) Establishment of new axenic hairy root lines by inoculation with *Agrobacterium rhizogenes*. Plant Cell Rep 7:9–12

Mugnier J, Musse B (1987) Vesicular-arbuscular mycorrhizal infection in transformed hairy root-inducing T-DNA roots grown axenically. Phytopathology 77:1045–1050

Mukherjee E, Sarkar S, Bhattacharyya S, Gantait S (2020) Ameliorated reserpine production via *in vitro* direct and indirect regeneration system in *Rauvolfia serpentina* (L.) Benth. ex Kurz. 3Biotech 10: 294

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–495

Nedelkoska TV, Doran PM (2001) Hyperaccumulation of nickel by hairy roots of alyssum species: comparison with whole regenerated plants. Biotechnol Prog 17:752–759

Nishikawa K, Ishimaru K (1997) Flavonoids in root cultures of *Scutellaria baicalensis*. J Plant Physiol 151:633–636

Nunes IS, Faria JMS, Figueriredo AC, Pedro LG, Trindade H, Barroso JG (2009) Menthol and geraniol biotransformation and glycosylation capacity of *Levisticum officinale* hairy roots. Planta Med 75:387–391

Orden AA, Bisogno FR, Cifuente DA, Giordano OS, Sanz MK (2006) Asymmetric bioreduction of natural xenobiotics by *Brassica napus* hairy roots. J Mol Catal B Enzym 42:71–77

Osama K, Pallavi S, Pandey AK, Mishra BN (2013) Modelling of nutrient mist reactor for hairy root growth using artificial neural network. Eur J Sci Res 97:516–526

Palavalli RR, Srivastava S, Srivastava AK (2012) Development of a mathematical model for growth and oxygen transfer in *in vitro* plant hairy root cultivations. Appl Biochem Biotechnol 167:1831–1844

Palazon J, Cusido RM, Bonfill M, Mallol A, Moyano E, Morales C, Pinol MT (2003a) Elicitation of different *Panax ginseng* transformed root phenotypes for an improved ginsenoside production. Plant Physiol Biochem 41:1019–1025

Palazon J, Moyano E, Cusido RM, Bonfill M, Oksman-Caldentey K-M, Pinol MT (2003b) Alkaloid production in *Duboisia* hybrid hairy roots and plants overexpressing the *h6h* gene. Plant Sci 165:1289–1295

Pandey P, Kaur R, Singh S, Chattopadhyay SK, Srivastava SK, Banerjee S (2014) Long-term stability in biomass and production of terpene indole alkaloids by hairy root culture of *Rauvolfia serpentina* and cost approximation to endorse commercial realism. Biotechnol Lett 36:1523–1528

Panigrahi J, Dholu P, Shah TJ, Gantait S (2018) Silver nitrate-induced in vitro shoot multiplication and precocious flowering in *Catharanthus roseus* (L.) G. Don, a rich source of terpenoid indole alkaloids. Plant Cell Tissue Organ Cult 132:579–584

Parizi KJ, Rahpeyma SA, Pourseyedi S (2020) The novel paclitaxel-producing system: establishment of *Corylus avellana* L. hairy root culture. In Vitro Cell Dev Biol—Plant 56:290–297

Patra N, Srivastava AK (2015) Use of model-based nutrient feeding for improved production of artemisinin by hairy roots of *Artemisia annua* in a modified stirred tank bioreactor. Appl Biochem Biotechnol 177:373–388

Payne J, Hamill JD, Robins RJ, Rhodes MJC (1987) Production of hyoscyamine by "hairy root" cultures of *Datura stramonium*. Planta Med 53:474–478

Peng CX, Gong JS, Zhang XF, Zhang M, Zheng SQ (2008) Production of gastrodin through biotransformation of p-hydroxybenzyl alcohol using hairy root cultures of *Datura tatula* L. Afr J Biotechnol 7: 211–216

Piątczak E, Jeleńb A, Makowczyńskaa J, Zielińskac S, Kuźmaa Ł, Balcerczakb E (2019) Establishment of hairy root cultures of *Rehmannia elata* N.E. Brown ex Prain and production of iridoid and phenylethanoid glycosides. Ind Crop Prod 137:308–314

Piątczak E, Królicka A, Wielanek M, Wysokinska H (2012) Hairy root cultures of *Rehmannia glutinosa* and production of iridoid and phenylethanoid glycosides. Acta Physiol Plant 34:2215–2224

Preiszner J, Van Toai TT, Huynh L, Bolla RI, Yen HH (2001) Structure and activity of a soybean Adh promoter in transgenic hairy roots. Plant Cell Rep 20:763–769

Putalun W, Taura F, Qing W, Matsushita H, Tanaka H, Shoyama Y (2003) Anti-solasodine glycoside single-chain Fv antibody stimulates biosynthesis of solasodine glycoside in plants. Plant Cell Rep 22:344–349

Qin MB, Li GZ, Yun Y, Ye HC, Li GF (1994) Induction of hairy root from *Artemisia annua* with *Agrobacterium rhizogenes* and its culture *in vitro*. Acta Bot Sin 36(Suppl):165–170 (in Chinese with an English abstract)

Rahimi S, Hasanloo T, Najafi F, Khavari-Nejad RA (2012) Methyl jasmonate influence on silymarin production and plant stress responses in *Silybum marianum* hairy root cultures in a bioreactor. Nat Prod Res 26:1662–1667

Rajashekar J, Kumar V, Veerashree V, Poornima DV, Sannabommaji T, Gajula H, Giridhara B (2016) Hairy root cultures of *Gymnema sylvestre* R. Br. to produce gymnemic acid. In: Jain S (ed) Protocols for *in vitro* cultures and secondary metabolite analysis of aromatic and medicinal plants, Methods in Molecular Biology, vol 1391, second edn. Humana, New York

Ramakrishnan D, Curtis WR (2004) Trickle-bed root culture bioreactor design and scale-up: growth, fluid-dynamics, and oxygen mass transfer. Biotechnol Bioeng 88:248–260

Ranjan R, Ahmed N, Khanna R, Mishra BN (2009) Design of an ON/OFF mist duty cycle in mist bioreactors for the growth of hairy roots. Biotechnol Bioprocess Eng 14:38–45

Rawat JM, Bhandari A, Raturi M, Rawat B (2019) *Agrobacterium rhizogenes* mediated hairy root cultures: a promising approach for production of useful metabolites. In: New and future developments in microbial biotechnology and engineering: microbial secondary metabolites biochemistry and application. Elsevier B.V., pp 103–118

Renouard S, Corbin C, Drouet S, Medvedec B, Doussot J, Colas C, Maunit B, Bhambra AS, Gontier E, Jullian N, Mesnard F, Boitel M, Abbasi BH, Arroo RRJ, Lainé E, Hano C (2018) Investigation of *Linum flavum* (L.) hairy root cultures for the production of anticancer aryltetralin lignans. Int J Mol Sci 19:990

Rhodes MJC, Parr AJ, Giulietti A, Aird ELH (1994) Influence of exogenous hormones on the growth and secondary metabolite formation in transformed root cultures. Plant Cell Tissue Organ Cult 38:143–151

Rodriguez-Mendiola MA, Stafford A, Cresswell R, Aria-Castro C (1991) Bioreactors for growth of plant roots. Enzym Microb Technol 13: 697–702

Rodríguez-Llorente ID, Lafuente A, Doukkali B, Caviedes MA, Pajuelo E (2012) Engineering copper hyperaccumulation in plants by expressing a prokaryotic *copC* gene. Environ Sci Technol 46:12088–12097

Ron M, Kajala K, Pauluzzi G, Wang D, Reynoso MA, Zumstein K, Garcha J, Winte S, Masson H, Inagaki S, Federici F, Sinha N, Deal RB, Bailey-Serres J, Brady SM (2014) Hairy root transformation using *Agrobacterium rhizogenes* as a tool for exploring cell type-specific gene expression and function using tomato as a model. Plant Physiol 166:455–469

Rugini E, Mariotti D (1991) *Agrobacterium rhizogenes* T-DNA genes and rooting in woody species. Acta Hortic 300:301–308

Sajjalaguddam RR, Paladugu A (2016) Quercetin production from hairy root cultures of Indian mallow (*Abutilon indicum* L.). World J Pharm Pharm Sci 5:956–967

Samydurai P, Ramakrishnan R, Thangapandian V (2013) *Agrobacterium rhizogenes* mediated hairy root culture and genetic transformation of an endangered medicinal plant of *Decalepis hamiltonii* Wight & Arn. J Microbiol Biotechnol Food Sci 3:191–194

Sevon N, Hiltunen R, Oksman-Caldentey KM (1992) Chitosan increases hyoscyamine content in hairy root cultures of *Hyoscyamus muticus*. Pharm Pharmacol Lett 2:96–99

Sevon N, Oksman-Caldentey KM (2002) *Agrobacterium rhizogenes*-mediated transformation: root cultures as a source of alkaloids. Planta Med 68:859–868

Shahjahan A, Thilip C, Faizal K, Mehaboob VM, Raja P, Aslam A, Kathiravan K (2017) An efficient hairy root system for withanolide production in *Withania somnifera* (L.) Dunal. In: Malik S (ed) Production of plant derived natural compounds through hairy root culture. Springer, Cham, pp 133–143

Shanks JV, Morgan J (1999) Plant 'hairy root' culture. Curr Opin Biotechnol 10:151–155

Sosa Alderete LG, Talano MA, Ibañez SG, Purro S, Agostini E, Milrad SR, Medina MI (2009) Establishment of transgenic tobacco hairy roots expressing basic peroxidases and its application for phenol removal. J Biotechnol 139:273–279

Soudek P, Valenová S, Vavrikova Z, Vaněk T (2006) ^{137}Cs and ^{90}Sr uptake by sunflower cultivated under hydroponic conditions. J Environ Radioact 88:236–250

Spano L, Mariotti D, Cardarelli M, Branca C, Constantino P (1988) Morphogenesis and auxin sensitivity of transgenic tobacco with different complements of Ri T-DNA. Plant Physiol 87:479–483

Srivastava S, Srivastava AK (2006) Biological phosphate removal by model based continuous cultivation of *Acinetobacter calcoaceticus*. Process Biochem 41:624–630

Srivastava S, Srivastava AK (2012) Azadirachtin production by hairy root cultivation of *Azadirachta indica* in a modified stirred tank reactor. Bioprocess Biosyst Eng 35:1549–1553

Steingroewer J, Bley T, Georgiev V, Ivanov I, Lenk F, Marchev A, Pavlov A (2013) Bioprocessing of differentiated plant *in vitro* systems. Eng Life Sci 13:26–38

Sudha CG, Obul Reddy B, Ravishankar GA, Seeni S (2003) Production of ajmalicine and ajmaline in hairy root cultures of *Rauvolfia micrantha* Hook f., a rare and endemic medicinal plant. Biotechnol Lett 25:631–636

Sun J, Manmathan H, Sun C, Peebles CAM (2016) Examining the transcriptional response of overexpressing anthranilate synthase in the hairy roots of an important medicinal plant *Catharanthus roseus* by RNA-seq. BMC Plant Biol 16:108

Suresh B, Bais HP, Raghavarao KSMS, Ravishankar GA, Ghildyal NP (2005) Comparative evaluation of bioreactor design using *Tagetes patula* L. hairy roots as a model system. Process Biochem 40:1509–1515

Suresh B, Ravishankar GA (2004) Phytoremediation—a novel and promising approach for environmental clean-up. Crit Rev Biotechnol 24: 97–124

Swain SS, Rout KK, Chand PK (2012) Production of triterpenoid anticancer compound taraxerol in *Agrobacterium*-transformed root cultures of butterfly pea (*Clitoria ternatea* L.). Appl Biochem Biotechnol 168:487–503

Taya M, Yoyama A, Kondo O, Kobayashi T, Mitsui C (1989) Growth characteristics of plant hairy roots and their culture in bioreactors. J Chem Eng Jpn 22:89–94

Tayler BH, Amasino RM, White FF, Nester EW (1985) T-DNA analysis of plants regenerated from hairy root tumors. Mol Gen Genet 201: 554–557

Tepfer D (1984) Genetic transformation of several species of higher plants by *Agrobacterium rhizogenes*: sexual transmission of the transformed genotype and phenotype. Cell 37:959–967

Thakore D, Srivastava AK, Sinha AK (2015) Model based fed batch cultivation and elicitation for the overproduction of ajmalicine from hairy roots of *Catharanthus roseus*. Biochem Eng J 97:73–80

Thimmaraju R, Bhagyalakshmi N, Ravishankar GA (2004) In situ and ex situ adsorption and recovery of betalains from hairy root cultures of *Beta vulgaris*. Biotechnol Prog 20:777–785

Thimmaraju R, Bhagyalakshmi N, Narayan MS, Ravishankar GA (2003b) Kinetics of pigment release from hairy root cultures of *Beta vulgaris* under the influence of pH, sonication, temperature and oxygen stress. Process Biochem 38:1069–1076

Thimmaraju R, Bhagyalakshmi N, Narayan MS, Ravishankar GA (2003a) Food-grade chemical and biological agents permeabilize red beet hairy roots, assisting the release of betalaines. Biotechnol Prog 19:1274–1282

Thiruvengadam M, Praveen N, Kim EH, Kim SH, Chung IM (2014) Production of anthraquinones, phenolic compounds and biological activities from hairy root cultures of *Polygonum multiflorum* Thunb. Protoplasma 251:555–566

Toivonen L, Laakso S, Rosenqvist H (1992) The effect of temperature on hairy root cultures of *Catharanthus roseus*: growth, indole alkaloid accumulation and membrane lipid composition. Plant Cell Rep 11: 395–399

Toivonen L, Ojala M, Kauppinen V (1991) Studies on the optimization of growth and indole alkaloid production by hairy root cultures of *Catharanthus roseus*. Biotechnol Bioeng 37:673–680

Torregrosa L, Bouquet A (1997) *Agrobacterium rhizogenes* and *A. tumefaciens* co-transformation to obtain grapevine hairy roots producing the coat protein of grapevine chrome mosaic nepovirus. Plant Cell Tissue Organ Cult 49:53–62

Trulson AJ, Simpson RB, Shahin EA (1986) Transformation of cucumber (*Cucumis sativus* L.) plants with *Agrobacterium rhizogenes*. Theor Appl Genet 73:1–15

Tuan PA, Chung E, Thwe AA, Li X, Kim YB, Mariadhas VA, Al-Dhabi NA, Lee JH, Park SU (2015) Transcriptional profiling and molecular characterization of astragalosides, calycosin, and calycosin-7-O-β-D-glucoside biosynthesis in the hairy roots of *Astragalus membranaceus* in response to methyl jasmonate. J Agric Food Chem 63:6231–6240

Tusevski O, Stanoeva JP, Stefova M, Kungulovski D, Atanasova P, Sekulovski N, Panov S, Simic SG (2013) Hairy roots of *Hypericum perforatum* L.: a promising system for xanthone production. Central European J Biol 8:1010–1022

Tusevski O, Vinterhalter B, Krstić Milošević D, Soković M, Ćirić A, Vinterhalter D, Korać SZ, Stanoeva JP, Stefova M, Simic SG (2016) Production of phenolic compounds, antioxidant and

antimicrobial activities in hairy root and shoot cultures of *Hypericum perforatum* L. Plant Cell Tissue Organ Cult 128:589–605

Udomsin O, Yusakul G, Kraithong W, Udomsuk L, Kitisripanya T, Juengwatanatrakul T, Putalun W (2018) Enhanced accumulation of high-value deoxymiroestrol and isoflavonoids using hairy root as a sustainable source of *Pueraria candollei* var. *mirifica*. Plant Cell Tissue Organ Cult 136:141–151

Vanhala L, Eeva M, Lapinjoki S, Hiltunen R, Oksman-Caldentey KM (1998) Effect of growth regulators on transformed root cultures of *Hyoscyamus muticus*. J Plant Physiol 153:75–81

Verma SK, Gantait S, Jeong BR, Hwang SJ (2018) Enhanced growth and cardenolides production in *Digitalis purpurea* under the influence of different LED exposures in the plant factory. Sci Rep 8:18009

Vinterhalter B, Krstić-Milošević D, Janković T, Pljevljakušić D, Ninković S, Smigocki A, Vinterhalter D (2015) *Gentiana dinarica* Beck. hairy root cultures and evaluation of factors affecting growth and xanthone production. Plant Cell Tissue Organ Cult 121:667–679

Visser RGF, Jacobsen E, Witholt B, Feenstra WJ (1989) Efficient transformation of potato (*Solanum tuberosum* L.) using a binary vector in *Agrobacterium rhizogenes*. Theor Appl Genet 7–8:594–600

Wang F, Zhi J, Zhang Z, Wang L, Suo Y, Xie C, Li M, Zhang B, Du J, Gu L, Sun H (2017) Transcriptome analysis of salicylic acid treatment in *Rehmannia glutinosa* hairy roots using RNA-seq technique for identification of genes involved in acteoside biosynthesis. Front Plant Sci 8:787

Wetterauer B, Wildi E, Wink M (2018) Production of the anticancer compound camptothecin in root and hairy root cultures of *Ophiorrhiza mungos* L. In: Kumar N (ed) Biotechnological approaches for medicinal and aromatic plants. Springer, Singapore, pp 303–341

Wevar Oller AL, Agostini E, Talano MA, Capozucca C, Milrad SR, Tigier HA, Medina MI (2005) Overexpression of a basic peroxidase in transgenic tomato (*Lycopersicon esculentum* Mill. cv Pera) hairy roots increases phytoremediation of phenol. Plant Sci 169:1102–1111

Willmitzer L, Sanches Serrano J, Bushfels E, Schell J (1982) DNA from *Agrobacterium rhizogenes* is transferred to and expressed in hairy root plant tissues. Mol Gen Genet 186:16–22

Xu Z, Peters RJ, Weirather J, Luo H, Liao B, Zhang X, Zhu Y, Ji A, Zhang B, Hu S, Au KF, Song J, Chen S (2015) Full-length transcriptome sequences and splice variants obtained by a combination of sequencing platforms applied to different root tissues of *Salvia miltiorrhiza* and tanshinone biosynthesis. Plant J 82:951–961

Yamazaki M, Mochida K, Asano T, Nakabayashi R, Chiba M, Udomsin N, Yamazaki Y, Goodenowe DB, Sankawa U, Yoshida T, Toyoda A, Totoki Y, Sakaki Y, Castillo EG, Buell CR, Sakurai T, Kazuki S (2013) Coupling deep transcriptome analysis with untargeted metabolic profiling in *Ophiorrhiza pumila* to further the understanding of the biosynthesis of the anticancer alkaloid camptothecin and anthraquinones. Plant Cell Physiol 54:686–696

Yao SC, Bai L, Lan Z, Tang M, Zhai Y, Huang H, Wei R (2016) Hairy root induction and polysaccharide production of medicinal plant *Callerya speciosa* Champ. Plant Cell Tissue Organ Cult 126:177–186

Yaoya S, Kanho H, Mikami Y, Itani T, Umehara K, Kuroyanagi M (2004) Umbelliferone released from hairy root cultures of *Pharbitis nil* treated with copper sulfate and its subsequent glucosylation. Biosci Biotechnol Biochem 68:1837–1841

Zafari S, Sharifi M, Chashmi NA (2018) A comparative study of biotechnological approaches for producing valuable flavonoids in *Prosopis farcta*. Cytotechnology 70:603–614

Zhou L, Wang J, Yang C (1998) Progress on plant hairy root culture and its chemistry. 1. Induction and culture of plant hairy roots. Nat Prod Res Dev 10:87–95 (in Chinese with an English abstract)

Zhu C, Miao G, Guo J, Huo Y, Zhang X, Xie J, Feng J (2014) Establishment of *Tripterygium wilfordii* Hook. f. hairy root culture and optimization of its culture conditions for the production of triptolide and wilforine. J Microbiol Biotechnol 24:823–834